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THE MECHANISM OF VAGAL EFFECTS ON PULMONARY VENTILATION

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Since expiration involves relatively little muscular activity, and may be entirely passive (Gesell, 1936), the work of breathing is mainly inspiratory. Gradual filling of the lungs, in a normal inspiration, is accomplished by a muscular tetanus of progressively increasing strength. Consequently the rate of energy expenditure must increase throughout the act, and successive equal increments are added to inspiratory volume at increasing cost. Any increase of inspiratory depth, velocity remaining constant, entails extra work which is out of proportion to the added tidal volume (Rohrer, 1925). It is often assumed that the minute volume of air inspired measures the rate of activity of the respiratory center; but if this is true the activity of the center does not always conform to that of the respiratory muscles. Any given minute volume can be maintained by varying combinations of depth and frequency. A reduced frequency, however, cannot be offset by a reciprocal adjustment of depth without increasing the total energy expended.

Gad (1880) suggested that the motor discharge to the inspiratory muscles is proportional to the mean inspiratory volume rather than to ventilation rate. On a graph relating lung volume to time, the mean inspiratory volume in a given cycle is measured by the area included between the graph and the base line of expiration, divided by the duration of the cycle. Over a one-minute period the total discharge would be proportional to the area of an average cycle multiplied by frequency (referred to below as the "minute area"). This area in apneusis, as an extreme example, would obviously reflect the rate of inspiratory activity, and the stimulating effect of CO_2, somewhat better than the ventilation rate. For normal breathing, the validity of Gad's index appears to be confirmed in the recent studies

of Gesell, Magee and Bricker (1940) on the temporal distribution of inspiratory action potentials. They find, throughout inspiration, a steady recruitment of new motor units into activity, with some increase in the frequency of firing of those already active. The rate of discharge (number of unit potentials recorded per unit of time) rises along a gradient which corresponds to the rate of filling of the lungs. The mean rate of discharge, over any given period of time t, must then be approximately proportional to the mean volume over the same period; and the total discharge (mean rate $\times\ t$) proportional to mean volume $\times\ t$. The latter product is the area referred to above.

After vagal section the minute volume may fall (Hammouda and Wilson, 1932), particularly if the animal is already in a state of hyperpnea from rebreathing (Rice, 1938). Central stimulation of one vagus, so timed that it cuts short each inspiration near the normal level, reverses this effect. Respiratory frequency (Hillenbrand and Boyd, 1936) and minute volume (Hammouda and Wilson, 1939) are augmented. The latter authors infer that these changes of minute volume must result from parallel changes in the motor discharge; hence, that intermittent vagal stimulation actively excites the center and that vagal section removes a tonic excitatory influence. Since the altered ventilation is accompanied in each case by reciprocal changes of rate and depth, it seems to us, for the reasons outlined above, that the minute area might give more reliable information concerning the actual variations of inspiratory activity. We have therefore made comparative measurements of minute area and minute volume, from graphic records of our own and from those presented as typical by Hammouda and Wilson (1932, p. 86).

Procedure. Dogs were used, a combination of barbital-sodium (0.25 gram per kgm.) with pentothal sodium (15 mgm. per kgm.) being given intravenously. In 8 experiments intrapleural pressure was recorded instead of lung volume. Over a wide range inspiratory volume varies in direct ratio to negative pressure around the dog's lung (Cloetta, 1913), and the latter can be recorded photographically, avoiding the inertia to which nearly all forms of volume recording apparatus are subject in some degree. Through the chest wall was introduced one arm of an L-shaped glass tube, of 3 mm. bore. This part of the tube had several openings, and was adjusted to lie in the intrapleural space parallel to the ribs. The outside arm of the tube was connected to a segment capsule with a rubber membrane. In 4 experiments the dog (weighing 9 to 11.6 kgm.) was placed in an oblong box of about 98 liters capacity. The trachea was connected through its cannula to the outside air and the box sealed. The small pressure changes in the closed air space of the box, caused by and proportional to the respiratory changes of lung volume, were recorded optically.

The film records were enlarged to about 25 times their original area, and traced on paper. The base line of expiration was made continuous. The paper used (Keuffel and Esser graph, 358-14) was found to be of sufficiently uniform quality to permit comparative area measurements by the method of cutting out and weighing. Area measurements made by weight, on 20 squares of 25 sq. cm. each cut from separate sheets, gave a probable error of 0.17 sq. cm. On each record the depth of several consecutive inspirations was measured, and the average depth multiplied by frequency. The area was measured on the same graphs, and the average

TABLE 1

Comparison of minute volume and minute area, both expressed in arbitrary units (see text)

I, data from Hammouda and Wilson, 1932, figure 2 and accompanying table. II, data from figure 1 of this paper.

	FREQUENCY, PER MIN.	$N \times$ DEPTH, OR MINUTE VOLUME	MINUTE AREA
I			
	(n)		
A. Normal, before vagal section	12	100	100
B. Immediately after section	5	159	238
C. 30 minutes later	4.5	73	119
D. Later, effect of rebreathing	8	349	482
II			
A. Rebreathing, left vagus intact	32	100	100
After section of left vagus	17	64	193
B. Before vagal stimulation	6	100	100
During vagal stimulation	15	89	29
C. Before vagal stimulation	5.5	100	100
During vagal stimulation	10	123	71
D. Before vagal stimulation	7	100	100
During vagal stimulation	13.3	110	46

multiplied by frequency to give minute area. These data give minute volume and minute area in arbitrary units, the initial or control value being taken as 100 for comparison in the tables.

Results. a. *The effect of vagotomy.* Section of both vagi, if the animal is breathing quietly at the time from outside air without abnormally large dead space, is usually followed by an immediate increase of gross minute volume (confirming Rice, 1938, and Hammouda and Wilson, 1932, 1939). Later the minute volume is gradually reduced, reaching a new steady level. The minute area is also immediately augmented after vagotomy, relatively more than minute volume. Later the area also falls, but it always re-

mains, even after the new steady state is reached, above the pre-vagotomy figure. We have found no exceptions to this. It may be illustrated by our measurements on the graphs of Hammouda and Wilson (1932, p. 86), presented for comparison with their data on minute volume (table 1 of this paper). The total inspiratory discharge per minute evidently is augmented after vagotomy, even after the breathing has become stabilized. Minute volume fails to increase in proportion, because the deep and prolonged inspiration is relatively inefficient. The augmented inspiratory activity can be attributed simply to the loss of vagal inhibition. In quiet breathing the ventilation is initially increased, in spite of lowered efficiency. The intensity of chemical stimulation is thereby lowered, and this leads in turn to a reduction of inspiratory activity until a new equilibrium is reached.

If, on the other hand, the animal is already hyperpneic from rebreathing at the time of section, the minute volume falls immediately (confirming Rice, 1938), but the minute area nevertheless increases sharply (fig. 1, A, and table 1). The fall of ventilation is therefore not due to a reduced motor discharge, but to relatively ineffective grouping of an augmented discharge, as in apneusis.

b. *The effects of intermittent central stimulation of the vagus.* Brief tetanic stimuli, beginning at a fixed stage in each inspiration, were applied to the central stump of one vagus after section of both. The circuit was automatically controlled by the animal's breathing, in the manner described by Hillenbrand and Boyd (1936). The stimuli were of a strength just sufficient to cut short inspiration.

By this procedure, the frequency of breathing is regularly augmented (fig. 1, B, C, D). There is, however, a limit to the degree of acceleration thus attainable. When inspiration is cut short at a very early stage, acceleration is not enough to offset the lowered tidal volume, and minute volume is reduced (fig. 1, B, and table 1). If inspiration is allowed to reach a more advanced stage, the acceleration is somewhat less but minute volume is increased (fig. 1, C and D, and table 1). In either case, however, the minute area is reduced. The increase of minute volume results from more effective grouping of a diminished inspiratory discharge. The effect is similar, in this respect, to that of intermittent central stimulation of the vagus in apneusis (Pitts, Magoun and Ranson, 1939a).

In 3 of the series of 12 experiments stimulation of the vagus during inspiration occasionally failed to inhibit. Instead, there was an increase in velocity and depth of the current inspiration. One or two inspirations of a group were affected in this way, the others being cut short as usual. This inspiratory response was noted by Gesell and Moyer (1935). It is not abolished by local cooling of the nerve to 8°C., whereas the inhibition of inspiration, and the accelerator effect of intermittent stimulation, are

both lost (Hammouda and Wilson, 1939). Presumably, therefore, the irregular inspiratory reflex is mediated by a distinct fiber group, not concerned in the rate and ventilation effects here under consideration.

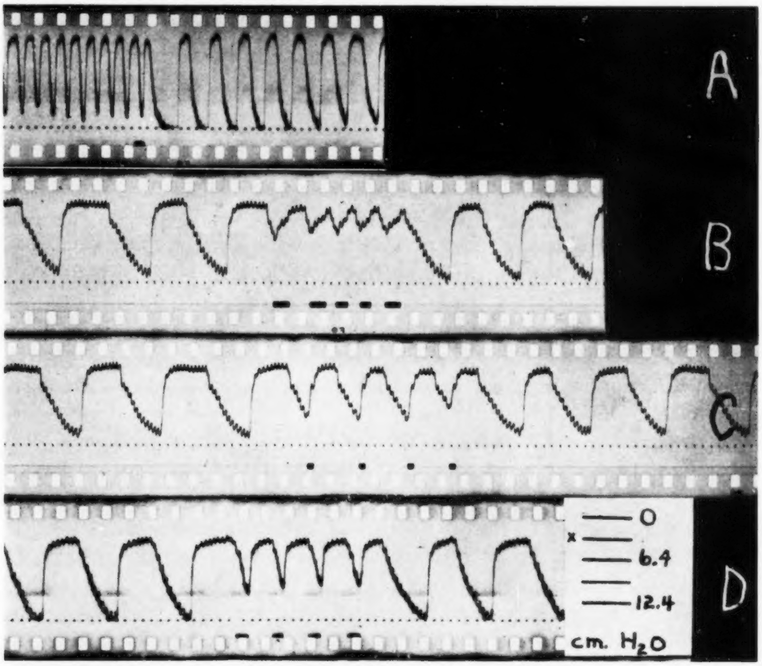

Fig. 1. Records of intrapleural pressure. Time in seconds. Calculation of data given in table 1.

A. Dog, 14.8 kgm., breathing through tube of 100 cc. capacity attached to tracheal cannula. Right vagus sectioned previously. At signal, section of left vagus. The first cycle following is obviously distorted by mechanical stimulation from section, and is not included in data tabulated.

B. Dog, 12.5 kgm. Both vagi sectioned. Signals, central stimulation of left vagus.

C. Same, stimulation applied at a later stage of inspiration.

D. Same animal as in A, later, after removal of extra dead space and stabilization of breathing. Signals, central stimulation of right vagus.

Discussion. With respect to vagal section, the present work merely confirms the contention of Gad (1880), that the act of inspiration becomes wastefully prolonged; and that the total activity of the respiratory motoneurones is invariably increased, without a corresponding increase of ventilation rate. This effect of vagotomy appears in hyperpnea as well

as in quiet breathing. The waste of energy may be exaggerated if inspiration is prolonged in time out of proportion to depth (as in fig. 1 A of this paper), but it occurs even if depth and duration are augmented in the same proportion. Normally, according to the calculations of Rohrer (1925) rate and depth are reciprocally adjusted in such a way that adequate ventilation is secured with a minimum expenditure of energy. This requires the finding of an optimum between rapid, shallow breathing, which is ineffective because of the dead space, and deep slow breathing, which is uneconomical.

The condition brought on by simple vagotomy appears to differ from apneusis only in degree. In the former condition the waste of inspiratory energy is limited, because inspiration is still cut short, though at a relatively advanced stage, by the pneumotaxic center (Stella, 1938a, 1938b; Pitts, Magoun and Ranson, 1939a, 1939b). When the pneumotaxic center also is eliminated, inspiration is further prolonged and still less efficient. In either condition, periodic central stimulation of a sectioned vagus, so timed as to imitate and replace the lost inflation reflex, can augment frequency and minute volume while reducing the total inspiratory discharge per minute.

The net influence of the vagi on the central mechanism is thus inhibitory, in the sense that it keeps the motor discharge tonically restrained. Gesell (1940a, 1940b) believes that the vagal inflation reflex accelerates the velocity of inspiration. He considers it to be purely excitatory, a positive drive exerted at first on the inspiratory motoneurones and abruptly transferred, at a certain stage of inspiration, to the expiratory side. When this shift takes place, inspiration is cut short by reciprocal inhibition. Admitting that such a mechanism is possible, the fact remains that the expiratory muscles contribute relatively little to the total energy expended in breathing, and the vagal expiratory drive can therefore add but little to the total motor discharge. In terms of the latter, the expiratory drive is far outweighed by the reciprocal inhibition of inspiration. And granting that the rate of inspiratory recruitment may be slower after vagotomy, the total inspiratory discharge, in each cycle and per minute, is nevertheless increased.

SUMMARY

1. The motor discharge to the inspiratory muscles, over a given period of time, is measured approximately by the mean inspiratory volume (Gad's index), or the area included between the base line of expiration and the graph relating lung volume to time.

2. Section of the vagi results in augmented inspiratory activity. This occurs no matter whether the animal is breathing quietly, or is hyperpneic from rebreathing, at the time of section. The impaired ventilation re-

sponse to CO_2, after vagotomy, is therefore due to a relative inefficiency of the act of inspiration, and not to a reduced motor discharge. The inefficiency results from loss of the inhibitory inflation reflex and a consequent wasteful prolongation of the inspiratory discharge in each cycle.

3. After vagotomy an intermittent stimulation of one vagus, cutting short each inspiration, augments frequency of breathing and may augment minute volume. The total inspiratory discharge per minute is at the same time reduced.

I wish to thank Mr. C. A. Maaske for assistance in the experiments.

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THE EFFECT OF GLYCOCOLL (GLYCINE) INGESTION UPON THE GROWTH, STRENGTH AND CREATININE-CREATINE EXCRETION IN MAN[1,2]

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Boothby (1) reported that the onset of muscular fatigue can be delayed by the addition of a considerable amount of glycine to the diet of a normal individual. He also demonstrated that in cases of myasthenia gravis the feeding of glycine tends to restore the wasted muscle tissue, thereby indicating an effect upon the physiological state of these tissues. Wilder (2) believed that glycine affected the creatinine excretion following vigorous physical exercise. Subsequently Ray, Johnson and Taylor (3) presented their findings in which they noticed that when a given adequate amount of gelatine (60 grams) was added to the normal diet of men there was an invariable increase in the amount of work produced before fatigue set in. The results reported varied from 37 per cent to 240 per cent increase above the training level.

Since Boothby (1) found that normal subjects were unable to tolerate large amounts of glycine without some discomfort, and since gelatine as a substitute proved to be cumbersome in the study of a large series, where convenience and portability were prime factors, it was felt that the administration of glycocoll (glycine) in tablet form would be the most ready way of taking this substance. In addition to the above factors, the form in which the glycocoll was to be administered was motivated by the fact that, to the author's knowledge, none of the above papers, concerned with muscular fatigue and glycine, reported quantitative studies on a group of subjects sufficiently large for statistical tests of reliability. Furthermore, the absence of controls, in the above studies, was felt to be an omission of an important magnitude.

The ready availability of glycocoll in tablet form (Tablets Glycolixir-

[1] Part of this research was carried out in the Department of Industrial Hygiene, Columbia University, Medical Center, New York, through the courtesy and coöperation of Dr. F. B. Flinn, Director.

[2] This study was made possible through the coöperation of E. R. Squibb & Sons, New York, in supplying the glycocoll in the form of Tablets Glycolixir-Squibb, and the control tablets of identical size, color and flavor minus the glycocoll.

Squibb) stimulated the undertaking of this study to determine what effect glycocoll (or glycine) had on growth (body weight, body height, and protoplasmic mass increase), upon endurance (ability to carry out sustained activity), on strength (muscular force), and upon the creatinine-creatine excretion following physical exertion in men.

EXPERIMENTAL. The subjects who volunteered for this investigation were for the most part freshmen taking the prescribed course in Hygiene at The City College, and athletes from the Division of Physical Education of the Department of Hygiene at the College. These subjects were males between the ages of 17 and 30. The subjects were athletes and non-athletes, who may or may not have been in training. Their normal daily routine was not modified in any respect, including diet, except in the partaking of the specific dosage of glycocoll or sugar. The selection of the subjects to act as controls and as experimentals was by random sampling.

Observations were made on one series of controls, numbering 19 students, taking placebo tablets of glucose and lactose (2 grams each tablet) at the rate of six tablets per diem for the duration of the experiment. Observations were also conducted on two series of experimental subjects, numbering 20 students each, taking 1 gram of glycocoll in tablet form (Tablets Glycolixir-Squibb) at the rate of six tablets per diem for the duration of the experiment which was ten weeks. It must be noted that the subjects were at no time aware as to whether they were taking glycocoll or sugar in as much as the tablets of glycocoll and the placebos were of the same size, shape, color, flavor, and were packaged the same way. The author was the only person who kept records as to the status of each subject. Each series had its initial and terminal measurements recorded. These measurements totalled 14 individual items. The grand total of observations on both the control series and the experimental series is 1632.

Tests and measurements. Each subject was given the following tests and examinations before and after the experimental period of 10 weeks:

1. Height—measured to the nearest $\frac{1}{4}$ of an inch.
2. Weight—measured to the nearest $\frac{1}{2}$ pound.
3. Protoplasmic mass—calculated from the formula:

$$PT = 55 \text{ per cent } FW + 25 \text{ per cent } AW$$

where PT is the protoplasmic tissue or mass; FW is the fatless weight, according to table 8—Jones (4); and AW is the actual weight in pounds.

4. Pulse rate per minute was counted for an interval of 15 seconds.
5. Blood pressure (systolic and diastolic) in millimeters was taken with the Tycos sphygmomanometer.
6. The Barach Index—determined from the formula given by Barach (5):

$$(SP \times PR) + (DP \times PR) = K$$

where SP is the systolic blood pressure, DP is the diastolic blood pressure, and PR is the pulse rate per minute.

7. Rogers Strength Index—calculated from the formula given by Rogers (6) for the physical capacity test by omitting the measurement of lung capacity, which was considered not necessary, and by substituting the McCloy formula (7) for the determination of arm strength, because the factors of height and weight are more equitably taken care of in the computation. This Modified Rogers Strength Index is derived from the formula:

> Grip strength (rt. hand) in pounds + grip strength (lt. hand) in pounds + back lift in pounds + leg lift in pounds + (1.77 × weight of the body + 3.52 × no. of chins − 46) = K

The bracketted portion of the formula represents the McCloy arms strength formula.

8. McCloy Endurance Index. Since there are few good tests of endurance requiring no period of preliminary training, because the devisors of the tests have measured interchangeably endurance and strength, it was hoped that the McCloy Track Test would serve the purpose of testing the possible effect of glycocoll on endurance. This test consists of running a 60 yd. dash, and when completely rested (at the end of 20 min.) a 220 yd. dash at full speed. The endurance ratio is the time for the 220 yd. dash divided by the time for the 60 yd. dash. The slower the time the higher the index, while the lower the index the greater the endurance.

9. Total Creatinine—determined by collecting a 6 hr. sample of urine following the accomplishment of all the tests, and determining the creatinine-creatine excretion as total creatinine concentration per 100 cc. according to the method of Benedict (8) using the Pulfrich Photometer.

Procedure for the measurement of strength. In calculating the strength index the following steps were carried out in using the apparatus consisting of *a*, an oval hand dynamometer; *b*, a back and leg dynamometer; *c*, a horizontal bar for the measuring of chinning or pull-ups, and *d*, parallel bars for the recording of dipping or push-ups:

1. Grip strength. The subject places the rounded edge of the dynamometer against the palm, with the indicator also toward the palm. The subject was permitted to assume any position with the arm or body (which was noted for the retest at the end of the experiment's duration), so long as the hand or fingers did not rest against the body or any other object. The recording was noted to the nearest pound.

2. Back lift. In determining the back lift, the subject stood at attention with hands on the frontal surface of the thighs. The observer then hooked the handle into the chain so that the top of the bar was just below the tips of the subject's fingers. The individual then bent forward at the hips and grasped the bar at the ends with one palm forward and one

palm backward. He, then, lifted steadily, but as vigorously as possible, and, after having exerted a maximum lift, released the pull slowly. The observer recorded the lift in pounds.

3. Leg lift. The position is the same as in the back lift. The dynamometer bar was placed across the thighs in the obtuse angle formed by the thigh and the trunk. The subject then was made to raise his head and chest and pull as hard as possible with legs and arms. This lift was then recorded in pounds.

4. Dipping or push-ups. The subject jumped to the cross rest with arms straight (this counted as one effort). He then lowered his body until the angle of upper arm and forearm was less than 90°, and then pushed up to

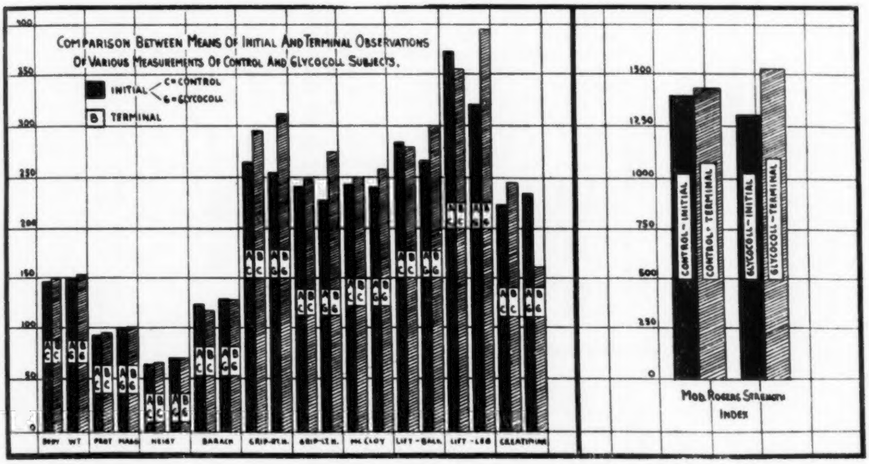

Fig. 1

the straight arm position. He repeated this movement as many times as possible.

5. Chinning or pull-ups. The subject's ability to raise and lower himself while hanging from a horizontal bar using the backward grip was recorded. No kick, jerk, or a "kip" motion was allowed. For both the push-ups and the pull-ups partial scoring was not included in the final calculations of the strength index.

Analysis of results and discussion. Figure 1 represents, in graphic form, the comparison between the means of the initial and terminal observations of both the control and the glycocoll series. Table 1 summarizes the results obtained for each one of the 14 separate tests carried out on the control series of 19 subjects and the experimental glycocoll series of 40 subjects at the start and finish of the experiment. The statistical analysis presented in this table is based on the following method of hand-

TABLE 1

Comparative analysis of the results obtained on a control series (19 subjects) and on an experimental glycocoll series (40 subjects), both series composed of normal, healthy, athletic and non-athletic young men of college age, with regard to each of 14 distinctive and separate tests

Control series: 19 subjects. Glycocoll series: 40 subjects

ANALYSIS	BODY WEIGHT	BODY HEIGHT	PROTOPLASMIC MASS	PULSE RATE PER MINTUE	BLOOD PRESSURE		BARACH INDEX	GRIP-STRENGTH		LIFT-STRENGTH		PUSH-UPS (DIPS)	PULL-UPS (CHINS)	MC CLOY ARMS STRENGTH INDEX	RUNNING		ENDURANCE INDEX	TOTAL CREATININE (CONC. IN MG. PER 100 CC.)	MODIFIED ROGERS INDEX
					Systolic	Diastolic		Right hand	Left hand	Back muscles	Leg muscles				60 yd. dash	220 yd. dash			
	lbs.	*inches*	*lbs.*					*lbs.*	*lbs.*	*lbs.*	*lbs.*				*secs.*	*secs.*			
Control series:																			
Mean (A) of initial observations	146.6	64.6	92.14	72.	110.	65.	126.	265.	241.	284.	367.	8.5	8.5	243.	8.5	32.5	3.84	226.	1400.
Mean (B) of terminal observations	148.4	64.8	93.47	71.	109.	67.	119.	294.	248.	281.	354.	8.5	9.6	250.	8.5	32.9	3.86	243.	1427.
Mean of the amount of change between initial and terminal observations	2.2	0.16	0.8	−1.9	1.	1.8	−1.6	29.	17.	0.3	−18.	0.0	1.0	7.3	0.9	0.4	0.01	17.	26.8
ϵ* of mean change	2.7	0.2	0.9	4.5	3.2	4.4	7.9	34.9	53.6	29.6	50.1	1.2	2.2	11.6	0.8	0.6	0.1	35.2	69.9
ϵ_M† of the mean change	0.633	0.045	0.211	1.063	0.760	1.045	1.873	8.216	12.622	6.966	11.805	0.272	0.512	2.737	0.199	0.138	0.027	8.300	16.482
Glycocoll series:																			
Mean (A_1) of initial observations	149.4	67.8	99.11	72.	110.	66.	130.	253.	229.	267.	322.	7.0	7.0	241.	8.7	32.9	3.80	235.	1312.
Mean (B_1) of terminal observations	152.7	67.9	100.20	71.	111.	68.	128.	310.	273.	299.	394.	8.0	9.0	256.	8.5	32.3	3.81	162.	1532.
Mean of the amount of change between initial and terminal observations	3.20	0.12	1.21	−1.95	0.25	1.00	−2.50	57.	53.	32.30	73.40	0.88	1.30	12.	−0.18	−0.57	0.005	−68.50	223.50
ϵ* of mean change	3.7	0.1	1.1	7.7	5.0	4.5	16.3	37.5	27.6	44.3	63.1	2.0	1.7	9.5	0.3	1.1	0.1	44.9	119.1
ϵ_M† of the mean change	0.859	0.034	0.247	1.720	1.126	0.997	3.654	8.382	6.186	9.916	14.113	0.449	0.373	2.129	0.056	0.246	0.030	10.054	26.640

Difference between means of change of Control and Experimental Series	1.00	0.04	0.41	0.05	0.75	0.80	0.93	28.00	36.00	32.00	91.40	0.88	0.30	4.70	1.08	−0.97	0.005	−85.50	196.70
‡Standard error of the above difference	1.068	0.056	0.325	2.022	1.357	1.450	3.720	11.740	14.060	12.120	18.400	0.270	0.630	2.860	0.210	0.280	0.030	13.040	29.700
Critical ratio: Observed diff./Standard error	0.9	0.71	1.26	0.02	0.55	0.55	0.25	2.38	2.56	2.64	4.97	3.22	0.47	1.64	5.22	3.44	0.16	6.56	6.62
Chances of obtaining a difference equal to or greater than the observed difference due to errors of random sampling	46 in 100	24 in 100	10 in 100	49 in 100	48 in 100	48 in 100	40 in 100	87 in 10,000	52 in 10,000	41 in 10,000	0 in 10,000	6 in 10,000	32 in 100	5 in 100	0 in 10,000	3 in 10,000	44 in 100	0 in 10,000	0 in 10,000

* ϵ = mean deviation = $\sqrt{\frac{\Sigma d^2}{N-1}}$. See Scott (13).

† ϵ_M = mean deviation of the mean = $\frac{\epsilon}{\sqrt{N}}$.

‡ Standard error of the difference between means = $\sqrt{(\epsilon_{M1})^2 + (\epsilon_{M2})^2}$. See Chaddock (14).

ling the data: *a*, the amount of *change* in each test for each individual was determined; *b*, the mean of the algebraic sum of these changes for all the individuals together was then calculated; *c*, the difference between the mean change for the control series and the mean change for the glycocoll series was arrived at; *d*, the standard error of this difference was then determined; *e*, the critical ratio was then calculated; and, *f*, the probability was then determined from the statistical tables of Holzinger (9).

From Table 1 it is apparent that the controls increased their body weight by 2.2 lbs. which is equal to a percentage change of 1.50 per cent. The glycocoll group increased their weight by 3.2 lbs. above starting level for a change of 2.14 per cent. The experimental group as a unit did not show a statistically significant increase in body weight since the probability is only 46 in 100.

Changes in body height observed in both the control and the glycocoll series were responsible for the slight changes noted in the protoplasmic mass, but were not significant.

The Barach test frequently used to confirm the results of strength and endurance tests proved to have no significance in this research.

In the matter of the grip-strength test, using the right hand, the control series improved after 10 weeks to the extent of 10.94 per cent. The glycocoll series exceeded their initial level by 22.53 per cent after a similar period. The difference between the means of the degree of change of the control and experimental series is 28.0 lbs., and the chances of obtaining a difference equal to or greater than this observed difference due to errors of random sampling are 87 in 10,000.

As to the grip-strength test, using the left hand, the controls improved to the extent of 17.0 lbs. or an approximate 5 per cent while the glycocoll group improved to the extent of 23.14 per cent. The standard error of the difference between the control and glycocoll means of change is 14.06 and the critical ratio is 2.56. In this case the deviation exceeded by more than twice the standard error and may thus be formally regarded as significant.

The results of the lift-strength test when using the back muscles showed that whereas the controls improved to the extent of 0.3 lb. the experimental series improved to the extent of 32.3 lbs. This difference of 32.0 lbs. between the groups is significant because the chances of obtaining a similar or greater difference due to errors of random sampling are 41 in 10,000. When using the leg muscles the glycocoll series improved by 22.79 per cent. The difference between the control and the glycocoll series in this phase of the lift-strength test has complete significance in favor of the glycocoll series because the critical ratio was found to be 4.97, which is practical certainty.

The cross rest on the parallel bars is a familiar exercise to develop the

extensors of the elbow (triceps) and depressors of the humerus (latissimus, teres major, deltoid 3). Measuring push-ups or dipping on the parallel bars showed that the glycocoll series improved, and this improvement was statistically significant.

When a person hanging by his hands tries to lift his body with his arms he brings into play the flexors of the elbow (biceps, brachialis, brachioradialis, pronator teres) and the depressors of the humerus. The improvement of the glycocoll series over the control series to the amount of 0.3 was not statistically significant.

The McCloy arm strength test proved to be statistically not significant. The results as regards the modified Rogers Strength Index show that there is definite significance to the amount of improvement in the glycocoll series. In this case the critical ratio is 6.62 which is practical certainty.

No attempt was made to determine the total volume of urine voided during the 6 hour period immediately following the series of 14 tests. The total creatinine excretion represented as a concentration in milligrams per 100 cc. showed, in the control series, an increase of 7.52 per cent in the terminal determination at the end of 10 weeks. A very striking effect of glycocoll is in evidence here for the experimental series showed a remarkable drop in the quantity of total creatinine excreted after physical exertion. After 10 weeks' ingestion of glycocoll, the experimental series' total creatinine concentration dropped to extent of 68.5 mgm. per 100 cc. This change proved to be practical certainty beyond any peradventure of doubt when subjected to the statistical analysis, since the critical ratio obtained was 6.56.

In this study it was hoped that endurance would be demonstrated objectively in the McCloy endurance test. However, that test did not force the subject to extend himself to the limit of his "staying" ability, and so did not elicit any significant change due to glycocoll ingestion. Certainly a more elaborate test of endurance that involves no preliminary education and practice in its performance might prove more efficacious in measuring this phenomenon.

In this study no attempt has been made to ascertain the mechanism by which glycocoll effects this increase in muscular strength and the extensive retention of creatine. The analysis of all the results obtained by using the statistical method of amount of change indicates that glycocoll has some definite beneficial effects, which are quite significant in view of the fact that no dietary restrictions were imposed upon the subjects undergoing the test. Invariably these same subjects reported that, above all else, they observed, subjectively, a marked improvement in their ability to sustain for a prolonged period of time any form of physical activity of a rather strenuous character. No such apparent increase in the resistance to fatigue was reported by the members of the control series. If we accept

the fact that hydrolysis of phosphocreatine is an essential part of the complex reactions which accompany the combustion of carbohydrate in muscle, then this reaction appears to be influenced to a great extent by glycocoll. Tripoli and Beard (10) have demonstrated the creatinogenic action of glycocoll, and Beard and Pizzolato (11) later suggested that glycine could be methylated to sarcosine in the synthesis of creatine. Rose, Ellis and Helming (12) had already confirmed the concept of storage of creatine in the bodies of male subjects. Thus the production and storage of creatine, as a whole, tends to prevent extreme changes in the acidity of the internal environment of the muscle cell because the change from phosphocreatine to orthophosphoric acid involves the substitution of a weak acid for a strong one (15). Apparently this reaction permits the elaboration of greater amounts of lactic acid without any alteration of the reaction in the muscle.

SUMMARY

A group of 19 subjects, acting as controls and ingesting for a period of 10 weeks fixed quantities of sugar, was compared with a group of 40 subjects, acting as experimentals and ingesting for the same time interval 6 grams of glycocoll per diem, as to 14 distinct and different measurements devised to evaluate achievement of physical strength and endurance, and to measure the amount of total creatinine excreted in the urine during a period of 6 hrs. following the physical exertion in the performance of the tests.

Under the influence of glycocoll a number of the tests revealed results that were definite improvements over the initial efforts. The grip strength improved by 22.5 per cent and 23.1 per cent for the right and left hand respectively. The lifting strength improved by 12.0 per cent and 22.8 per cent for the back and leg muscles respectively. The total body strength measured as the modified Rogers strength index showed an improvement of 17.0 per cent. The total creatinine excretion showed a drop of 29.2 per cent as compared with the initial excretion level.

The author desires to express his sincere thanks to Dr. Frederick B. Flinn, Director of the Department of Industrial Hygiene, Columbia University, for his coöperation in making it possible to have the urine samples analyzed for the creatinine-creatine content; to Dr. John M. Wilcox of E. R. Squibb & Sons for his interest and the supply of Tablets Glycolixir-Squibb and the placebos; and to Prof. John Dailey and Mr. Oberhofer of the Department of Hygiene, The City College, for their kindness and coöperation in permitting the canvass of volunteer subjects for this study.

This article would be remiss not to acknowledge with thanks the interest and diligence of Messrs. Henry Wittenberg and Sidney Malkin, graduate students of the Division of Physical Education, The City College, in

recording, as assistant observers, the results of the various tests performed by the subjects. To the many students who coöperated so earnestly in the experiment for the period of 12 weeks, the writer can only express his sincere appreciation and thanks.

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STUDIES ON CHOLINE ESTERASE ACTIVITY AND ACETYLCHOLINE CONTENT OF THE CENTRAL NERVOUS SYSTEM

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In spite of the fact that acetylcholine (ACh) affects the central nervous system (CNS) in minute quantities (Dikshit, 1934) the relation of functional changes in the CNS to the ACh content and the activity of the choline esterase (ChE) is not clear. Loewi (1937) reported that prolonged strychnine convulsions in frogs increased the ACh content of their CNS. Fegler and co-workers (1938–1939) found a decrease in total ACh in rabbit brain during strychnine convulsions, but a relative increase in free ACh. MacIntosh (1939) stated, however, that neither insulin hypoglycemia nor avitaminosis B had any effect on the ACh content of the brain. Pighini (1939) studied the ChE content of the brains of dogs and rabbits under conditions of tetanus intoxication and strychnine convulsions, but his data reveal no significant changes over the controls.

A simultaneous study of the ACh content and ChE activity of the CNS under a variety of conditions has been undertaken to determine possible changes in the concentration of either substance or in their relations to each other in states of altered brain function. The ACh content and ChE activity were determined in the brains of rabbits subjected to hypoglycemia, anoxia and metrazol, and in the brains of eserinized rabbits subjected to metrazol and strychnine convulsions. Further studies were made on the brains and spinal cords of frogs subjected to strychnine convulsions, heat coma and recovery from heat coma.

Methods. For the determinations of ACh the brain tissue was cut up in trichloracetic acid, the extract shaken with ether, the water layer evaporated under vacuum, and assayed for ACh on the eserinized rectus abdominis of the frog (Chang and Gaddum, 1933). ChE was determined by grinding the tissue with NaCl and determining the enzymatic activity of the supernatant fluid by the continuous electrometric titration method of Glick (1937) using 0.002 N NaOH over a period of thirty minutes.

In the rabbit experiments the animals were killed by a blow on the head,

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and the brain removed as quickly as possible. One half of the brain was used for the ACh determinations, the other half for the ChE determinations. Separate determinations were run on hemispheres and brain stems. In the frog experiments, two or three animals were pooled for the ACh determinations. The ChE determinations were made on single frogs. In both cases, separate determinations were run on brains and spinal cords.

The rabbits were unanesthetized. Anoxia was produced by inhalation of 7 per cent oxygen from Douglas bags (Gellhorn and Packer, 1940). Insulin[2] was injected subcutaneously; metrazol, eserine and strychnine were administered intravenously. In every instance, rabbits which received eserine were first injected with 1 mgm./kgm. of atropine intravenously.

TABLE 1

Choline esterase activity and acetylcholine content of rabbits' brain under conditions of insulin hypoglycemia and oxygen lack

NUMBER OF ANIMALS	TREATMENT	HEMISPHERE		BRAIN STEM	
		Choline esterase activity, cc. 0.002 N NaOH/g.	Acetylcholine content, γ ACh/g. tissue	Choline esterase activity, cc. 0.002 N NaOH/g.	Acetylcholine content, γ ACh/g. tissue
8	Control	21.74 (19.50-23.80)	1.5 (1.0-2.0)	29.36 (19.53-37.98)	2.3 (2.0-2.7)
6	Insulin, 0.5 U-/kgm.	20.27 (18.83-24.64)	1.3 (1.1-1.7)	31.91 (24.17-37.22)	2.1 (1.9-2.4)
3	Anoxia, 7%—2 hrs.	25.61 (22.97-28.00)	1.6 (1.4-1.9)	29.00 (23.46-34.35)	2.2 (2.1-2.4)
2	Insulin and metrazol (30 mgm.-/kgm.)	20.45 (19.73-21.07)	1.4 (1.2-1.5)	28.11 (27.52-28.70)	2.2 (2.1-2.2)

RESULTS. In the first series of experiments, six rabbits were made hypoglycemic by the injection of 0.5 U per kgm. of insulin, three rabbits were subjected to 7 per cent O_2 for two hours, and two rabbits were given 5U per kgm. of insulin followed 4 hours later by 50 mgm. per kgm. of metrazol. The values for ACh and ChE were compared with those of eight normal rabbits (table 1). In none of the experimental animals was there any change in the ACh content or ChE activity as compared with the controls.

A second series of experiments was run on eight rabbits subjected to eserine, two rabbits given eserine plus metrazol and four given eserine plus strychnine (table 2). Five of the eserinized rabbits were killed at

[2] Kindly supplied by Eli Lilly and Company.

the end of five minutes, and it was found that there was a marked suppression of ChE activity in both hemisphere and brain stem. The ACh content of both parts of the brain was increased. Three of the rabbits were killed after approximately an hour, at which time the ChE had returned to essentially normal values, while the ACh was still somewhat high. These experiments give proof that eserine injected into animals produces a reversible inhibition of ChE *in vivo*, accompanied by an increase in the ACh level. Eserinized rabbits made convulsive with metrazol or strychnine and killed after five minutes showed no differences in the ACh content or ChE activity of either hemisphere or brain stem from animals given eserine alone.

TABLE 2

Choline esterase activity and acetylcholine content of rabbits' brain following intravenous eserine and eserine plus convulsants

NUMBER OF ANIMALS	TREATMENT	HEMISPHERE		BRAIN STEM	
		Choline esterase activity*	Acetylcholine content, γ*	Choline esterase activity	Acetylcholine content, γ
8	Control	21.74 (19.50-23.80)	1.5 (1.0-2.0)	29.36 (19.53-37.98)	2.3 (2.0-2.7)
5	Eserine, 0.25-0.75 mgm./kgm., 5 min.	10.68 (7.41-16.49)	2.5 (2.0-3.0)	12.74 (8.97-18.28)	3.0 (2.9-3.0)
3	Eserine, 0.75-1.5 mgm./kgm., 49 min.	18.69 (16.14-20.80)	2.1 (1.9-2.3)	26.55 (20.93-32.90)	2.8 (2.3-3.0)
2	Eserine, 0.5 mgm./kgm., 5 min.; metrazol, 30 mgm./kgm.	8.63 (7.94-9.31)	2.4 (2.1-2.6)	12.22 (11.76-12.68)	3.1
4	Eserine, 0.5 mgm./kgm., 5 min.; strychnine, 0.4 mgm./kgm.	10.40 (7.34-13.19)	2.9 (2.2-3.4)	11.25 (8.84-13.73)	3.2 (2.6-3.4)

* As expressed in table 1.

As there were no changes produced in the ACh content or ChE activity in the brains of warm blooded animals except in the experiments involving eserine, a third series of experiments was performed on frogs, which could be subjected to more prolonged changes in function of their central nervous system. Excitation was produced by strychnine convulsions, which were allowed to continue for four hours. Table 3 gives the results of these

experiments. Both the brain and spinal cord of strychninized frogs showed an increase in ACh content over the control frogs. The average value for the ChE activity was the same for both groups of animals. The results on the increase in ACh content confirm those of Loewi (1937).

TABLE 3

Choline esterase activity and acetylcholine content of frogs' central nervous system following prolonged strychnine convulsions

TREATMENT	SPINAL CORD				BRAIN			
	Experiments	Choline esterase activity*	Experiments	Acetylcholine content, γ*	Experiments	Choline esterase activity	Experiments	Acetylcholine content, γ
Control	12	87.18 (71.92–106.47)	6	4.5 (4.4–4.7)	8	40.83 (28.14–47.85)	5	5.8 (5.1–6.8)
Strychnine, SO_4, 0.1 mgm.	12	87.11 (66.67–117.58)	6	6.2 (4.8–7.1)	8	36.36 (28.79–51.67)	6	7.2 (6.5–8.5)

* As expressed in table 1.

TABLE 4

The effect of heat coma and recovery from heat coma on the acetylcholine content and choline esterase activity of frogs' central nervous system

TREATMENT		SPINAL CORD				BRAIN			
		Experiments	Choline esterase activity, cc. 0.002 N NaOH/g.	Experiments	Acetylcholine content, γ ACh/g. tissue	Experiments	Choline esterase activity, cc. 0.002 N NaOH/g.	Experiments	Acetylcholine content, γ ACh/g. tissue
Controls		12	87.18 (71.92–106.47)	6	4.5 (4.4–4.7)	8	40.83 (28.14–47.85)	5	5.8 (5.1–6.8)
Heat coma	A*	5	36.76 (30.64–46.29)	4	3.1 (3.0–3.3)	5	19.31 (13.86–24.25)	5	4.5 (4.0–4.9)
	B†	4	60.81 (49.02–70.00)	3	5.4 (4.4–5.9)	4	31.52 (28.94–33.00)	3	5.9 (5.2–6.6)

* A, frogs subjected to 15 minutes of heat at 37–39°C. and killed immediately thereafter.

† B, subjected to 15 minutes of heat at 37–39°C., and killed two days later, when recovery had taken place.

In regard to the ChE activity, however, Nachmansohn (1938) reported that strychnine has a definite inhibitory action on ChE activity in *in vitro* experiments, and he calculated that the quantity of strychnine used to produce convulsions in frogs is sufficiently high to produce ChE inhibition *in vivo*, although he did not perform any such experiments.

Inhibition of activity of the central nervous system of frogs was produced by keeping the frogs at temperatures of 37 to 39°C. for fifteen minutes, thus producing a comatose condition. Analysis of the ACh content and ChE activity of the brain and spinal cord of these frogs showed a depression of ChE activity and a decrease in ACh content as compared to the control frogs (table 4). A second group of frogs was allowed to recover from the heat coma, and their CNS assayed two days later for ACh and ChE, at which time their reflexes were normal. The ACh content had returned to normal, and the ChE activity, although the values were higher than in the comatose frogs, had not quite returned to the control level.

Discussion. Although these experiments offer no conclusive evidence for or against the rôle of ACh in the transmission of impulses across the synapses within the central nervous system, they show that in the warm blooded animal the esterase may be inhibited *in vivo* by 50 per cent or more, with consequent increase in the ACh content of the central nervous system without grossly disturbing its function. Furthermore, they demonstrate that in cold blooded animals (possibly on account of the more severe procedures?) varying degrees of central excitability are associated with similar and reversible changes in the ACh content of the nervous system.

SUMMARY

1. Experiments on unanesthetized rabbits show that neither prolonged anoxia, hypoglycemia nor convulsions alter the acetylcholine content and choline esterase activity of hemispheres and brain stem.

2. Intravenous injection of eserine (0.5 mgm./kgm.) markedly inhibits choline esterase activity and increases the acetylcholine content of the brain. The effects are reversible with time.

3. Neither convulsions nor oxygen lack alter the acetylcholine and the choline esterase activity in eserinized rabbits.

4. Experiments on frogs show that strychnine convulsions are accompanied by a marked increase in the acetylcholine content of the brain and spinal cord. There is no significant alteration in the choline esterase activity.

5. When frogs are warmed to 38°C. or higher, a comatose condition appears. The central nervous system of such animals shows a decrease in both acetylcholine content and choline esterase activity. When the heated frogs are allowed to recover, they show, 48 hours later, together with restoration of nervous system functions, a return of choline esterase activity and acetylcholine content to approximately normal values.

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TRANSFER OF RADIOACTIVE SODIUM ACROSS THE PLACENTA OF THE GUINEA PIG[1]

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A considerable number of investigators (15, 17, 19) interested in problems related to placental permeability have emphasized that in this field there is an almost complete lack of quantitative data. This is due to the absence in the past of a suitable method of investigation. Observations on the placenta of a preliminary nature (7, 8) with radioactive sodium have demonstrated that the use of isotopes provides the direct and relatively simple approach demanded by the problem.

The present project has a double goal. The first is to study transfer across the placenta throughout the gestation period, so far as is feasible, and to correlate the resulting data with important related phenomena such as the growth rate of the fetus. The second is to widen these studies to include the several morphological types of placentae. In the end, consequently, it is hoped to have established a foundation for the comparative physiology of placental transfer.

Radioactive sodium ion, present as NaCl, has been chosen as the beginning tracer material for several reasons. It is easily prepared by deuteron bombardment of NaCl, it has a favorable half life, its behavior in the body is not complex, and it meets the requirement of being a strictly physiological substance.

Apparatus and methods. A. *Apparatus for measurement of radioactivity.* In the present work a pressure-ionization chamber (1) coupled to a Wülf or Edelmann type single-fiber electrometer (21) was used. The apparatus is moderately inexpensive, quite rugged, highly precise, and sensitive.

In practice the radioactive material is placed in a cup at a geometrically reproducible position below the pressure-ionization chamber (fig. 1). The pressure-ionization chamber consists of a brass chamber of large volume (about 1500 cc.) with a window at its base made of aluminum foil 0.1 mm. thick. It is filled with N_2-gas under 25 lbs.' pressure. Within

[1] Aided by a grant from the Rockefeller Foundation Fluid Research Fund of the School of Medicine, the Johns Hopkins University.

the chamber is a space enclosed by a charged, wire screen maintaining a potential gradient of roughly 40 volts per centimeter across the space to the central collecting rod. Radiation from the radioactive material produces ions in its path. The positive ions so created, and within the space enclosed by the high-potential screen, are moved by the potential gradient to the central collecting rod which is insulated with amber. As the ions are deposited the insulated rod becomes charged, and the charge spreads over a shielded lead to the platinum-coated quartz fiber or string of the electrometer. (Platinum-coated quartz fibers have been found superior to Wollaston wires in the Wülf electrometer.)

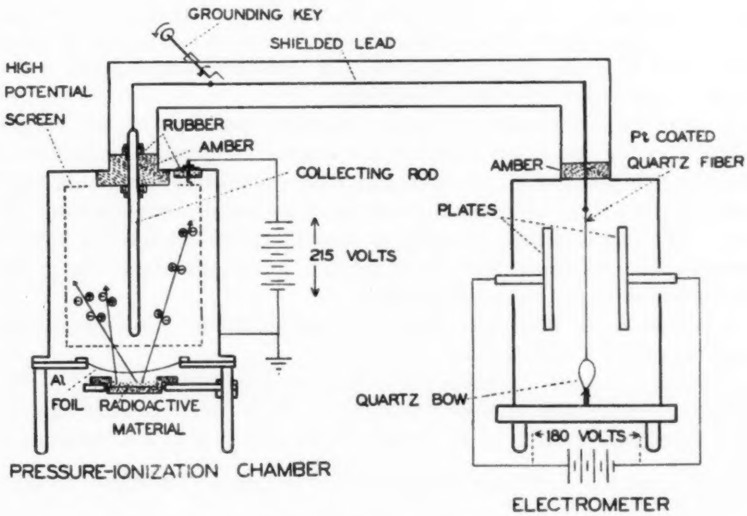

Fig. 1. Schematic diagram of pressure-ionization chamber and electrometer circuit.

The quartz fiber is suspended between two plates. Established between these plates is a potential gradient of about 180 volts per centimeter so that the fiber moves between these plates when it acquires a charge. The rate of movement of the fiber is observed through a microscope with a micrometer eyepiece and timed with a stopwatch. Since the charging rate, the strength of the radioactive sample, and the rate of movement of the fiber are all directly proportional, it is sufficient to measure the rate of movement to determine the sample's strength. The instrument is always calibrated with a uranium standard. A grounding key serves to shunt off the charging current to the fiber when the apparatus is not in use.

There are a number of electrometers and vacuum tube circuits (4, 6,

21) which have been proposed for use with an ionization chamber. The Dersham and Lindemann electrometers possess a peculiar and unexplained drift effect peculiar to the needle torsion type instrument (14). Because of this, these electrometers have a scale deflection which varies considerably from linearity with charging rate and makes this type of instrument undesirable for the purpose at hand (7 separate instruments were tested).

The electrometer circuit is very sensitive to temperature change and consequently the apparatus is placed in a constant temperature box.

Measurement of radioactivity with the pressure-ionization chamber and string electrometer presents the following sources of error:

1. Non-linearity of the electrometer response when plotted against sample strength. This is minimized by careful construction of the electrometer, particularly with respect to the amber insulation and quartz fiber, and by using the electrometer, at constant temperature, within ranges safely below its point of instability.

2. Geometric variability. Unless successive positions of the samples relative to the ionization chamber are exactly comparable, considerable error may be introduced. In this work care was taken to distribute liquid and ash samples evenly over the bottom of a special paper-lined bakelite cup. The cup was then placed in a mechanical mount which assured a reproducible position of the sample relative to the window of the ionization chamber at time of measurement.

3. Vibration of the fiber. This was minimized by providing a firm mounting for the instrument.

4. Battery voltage fluctuations. These are important only in the electrometer circuit and at high sensitivity. Here the sensitivity doubles with voltage increments of the order of 0.05 volt. This is a variation of $\frac{0.05 \text{ volt}}{180 \text{ volts}}$ or 2.5 parts per 10,000 in the voltage. Therefore accurate thermostatic control and careful use of the batteries is necessary.

5. Statistical fluctuations. Rate measurements involving low numbers of particles entering the chamber are affected by the statistical fluctuation of the numbers of particles entering the chamber. If a precision of "ϵ" in the expression of the rate is desired, at least $\frac{1}{\epsilon^2}$ particles must be counted, e.g., if $\epsilon = 1$ per cent, $\frac{1}{\epsilon^2} = \frac{1}{(0.01)^2} = 10{,}000$ particles must be counted in order to state the rate with a probable error of not more than 1 per cent.

6. Background. This is the minimal deflection rate of the instrument and is due to stray radiations entering the ionization chamber, to battery voltage fluctuations, etc. It is rather constant in magnitude and must be accounted for in the deflection rate read during measurement of a radioactive sample. In our apparatus, with its 1500 cc. chamber, background

is equivalent to a beta-ray source emitting 15 betas per second in all directions and placed at the standard position occupied by a sample.

7. Absorption of the radiation. Absorption by the samples is variable and must be accounted for. This has conveniently been done with the aid of experimentally established correction curves (fig. 2) relating the amount of the material to its absorption. The volume of liquid samples (plasma) taken for measurement was always 2 cc.; the correction factor for these samples was 1.32.

8. Radioactive decay. This may be corrected by means of the equation for radioactive decay:

$$\log\left(\frac{N}{N_0}\right) = \frac{-0.3010\ t}{\lambda}$$

where N/No is the fraction of radioactive atoms left after time t and λ is the half life of the particular radioactive material.

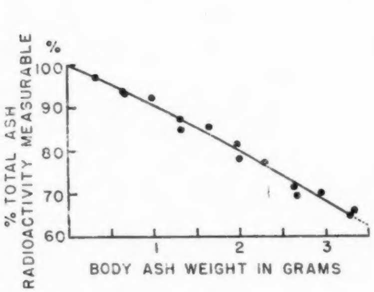

Fig. 2

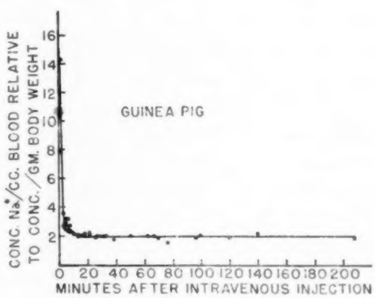

Fig. 3

Fig. 2. Absorption of radiation from known quantities of Na^{24} by varying amounts of body ash of rats. The cup containing the ash had a diameter of 5 cm.

Fig. 3. Rate of equilibration of Na^{24} (injected intravenously) with the body fluids.

9. Adsorption. It was found that the bakelite cups retained appreciable activity of samples placed in them, even after careful washing. The problem was answered by the use of replaceable waxed paper linings for the cups.

10. Miscellaneous errors. These may arise from contamination of samples or measuring apparatus with radioactive materials, especially those of long half life, accidentally spread in the laboratory; from transfer losses in handling the samples; and from such inaccuracies as are present in timing the interval of fiber movement, evaluating readings on the micrometer scale, etc.

The overall precision of the radioactivity measurements attained with usual sample strengths (100 to 2000 counts per second) is about 4 per cent,

but in rare cases where samples are only a fraction of background (3 to 5 betas per second) the precision may be no more than 40 per cent.

Preparation of samples. Radioactive sodium (Na^{24}) was prepared by means of the electrostatic pressure generator of the Department of Terrestrial Magnetism, Carnegie Institution of Washington, as previously described (8). The sodium chloride targets were used for an experiment at least 24 hours after preparation to allow time for the disappearance of any contamination of radioactive Cl^{38}.

About 40 mgm. of NaCl dissolved in 2 cc. of warm water were used for each of the experiments. This salt had an activity of between 1 and 2 microcuries in all the experiments except those where fetal size was very small; in these occasional instances, as high as 5 microcuries were used. The maximal doses used were well below radiation tolerance limits (5,11,18).

Units. Activity of the radioactive material is expressed in terms of beta-particles per second, e.g., a sample of strength 100 beta-rays per second in our apparatus would be one that gave a deflection rate equivalent to a beta-ray point source emitting 100 beta-rays in all directions and situated at the standard measuring position in our apparatus. One microcurie is about 37,000 particles per second in these units.

All the results on rate of transfer of Na^{24} to the fetus have involved measurement of the radioactivity of the fetus and a corresponding sample of maternal plasma. For comparison of the results of the different experiments, the concentration of Na^{24} found in the fetus has been divided by that found in 1 cc. of maternal plasma. The term, "corrected," frequently used in presentation of the results therefore means that the amount of Na^{24} found in the fetus has been corrected to a concentration of one beta-particle per cubic centimeter maternal plasma. These units will be adhered to in subsequent papers. To change data on transfer of Na^{24} across the cat placenta, given in a preliminary report (7), to this basis, they must be multiplied by two, as they were based on a unit concentration of one beta per 2 cc. plasma.

Procedure with animals. Nine experiments were performed to follow changes with time in blood concentration of Na^{24} after intravenous injection. Large, adult guinea pigs were chosen. Under nembutal anesthesia, a cannula was introduced into the proximal end of the carotid artery. At zero time, Na^{24} in isotonic solution containing heparin was injected into an arm or leg vein. Samples of blood of 1 or 2 cc. were then taken from the carotid cannula and measurement made of their radioactivity.

Pregnant guinea pigs, totaling 30, formed the bulk of the experimental material. They were obtained from several independent sources and so were not of a uniform strain. The experimental procedure with all of these animals was alike. They received an intravenous injection of radioactive

salt under light ether anesthesia. In the rat (8) such anesthesia has been found to be without demonstrable effect on placental transfer. Anesthesia was immediately discontinued after the injection. After a time interval fixed by the purpose of the experiment, the animal was again etherized, the fetuses delivered by Caesarian section, and immediately thereafter a sample of heart's blood taken from the mother. The fetuses were weighed with a precision of about 5 per cent after removal of the fetal membranes (with fetuses of low weight several were pooled), ashed at red heat with sulphuric acid, the ashes weighed, and the radioactivity of the maternal blood plasma and ashed fetal remains then determined. The placenta was separated from the decidua basalis and weighed after superficial blood had been absorbed by filter paper.

RESULTS. *Rate of escape of Na^{24} from blood plasma.*[2] The concentration levels of Na^{24} in the blood relative to that in a unit body weight at various periods after intravenous injection are shown in figure 3. Curves representing the fall in blood Na^{24}-concentration with time were obtained for nine animals. To secure a clear, averaged picture of events the several curves were fitted on a common graph (fig. 3) by multiplying each by a factor which served to bring their equilibrium values in close agreement. It is to be expected that within a short interval following injection, thorough mixing of Na^{24} with the blood did not take place and consequently the Na^{24}-concentrations at these times lose precision in their meaning. For example, assuming blood volume to be 10 per cent of body volume, the initial concentration of Na^{24} per cubic centimeter blood relative to that per gram body weight would be equal to 10 units on the graph. A value as high as 14 has, however, been found one minute after injection.

For the present purposes, the important observations are that the concentration of Na^{24} in the blood falls to a value within 90 per cent of its equilibrium value in 5 minutes, and remains constant for over 3.5 hours afterwards. This is in substantial agreement with the findings of Hevesy (12) on the rabbit.

The data of figure 3 can be used to calculate the volume of total extracellular fluid. Such a calculation gives the result that the extracellular fluid is 25 per cent of the weight of the animal on the assumption that sodium is distributed only in the extracellular fluid, and that plasma volume is 50 per cent of total blood volume. This value compares favorably with that found by the same (9) or analogous methods (20).

Establishment of equilibrium between maternal plasma and fetus. In order to plan experiments to determine the rate of transfer of Na^{24} across the placenta, it was necessary to know the shape of the curve describing the establishment of equilibrium between fetus and maternal plasma. In these experiments, all members of a litter were delivered as nearly

[2] The symbols Na^{24} and Na are used for the respective ions.

simultaneously as possible; obvious changes in the state of the uterus made any other procedure unacceptable. This observation was substantiated by the finding that delayed second deliveries made 30 minutes or more after the first gave placental transfer rates as much as 70 per cent lower than in the undisturbed uterus.

The data of figure 4 indicate that equilibrium is reached in fetuses of 60 grams or greater in from 5 to 7 hours. The concentration of Na^{24} in the fetus appears to increase linearly for 2 or 3 hours after intravenous injection into the mother. Measurements of the increase in concentration of Na^{24} up to this time consequently form a reliable criterion of the rate of transfer of Na^{24} across the placenta. In view of these findings, the routine

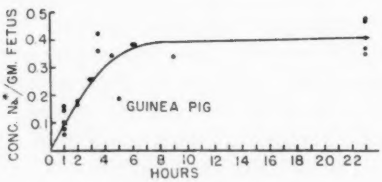

Fig. 4

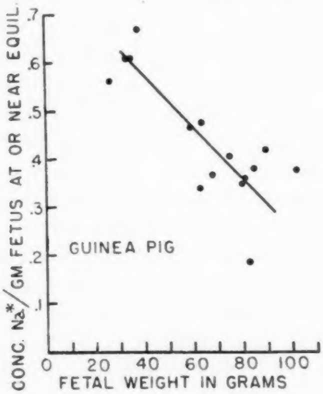

Fig. 5

Fig. 4. Rate of equilibration of Na^{24} in the maternal plasma with that of the fetus. All of the fetuses for these experiments weighed more than 60 grams. The concentration of Na^{24} in the fetus has been corrected to a concentration of one beta-particle per cubic centimeter maternal plasma as explained in the section above on "units."

Fig. 5. Variation of equilibrium concentration of Na^{24} with fetal size. The concentration of Na^{24} in the fetus has been corrected to a concentration of one beta-particle per cubic centimeter maternal plasma.

procedure for determination of placental transfer rates has been to remove fetuses 1 hour after injection of Na^{24} into the maternal circulation.

The magnitude of the concentration of Na^{24} reached at equilibrium is a function of fetal size, being greater in small than in large fetuses. This is shown in figure 5. From these equilibrium values, the approximate volume of extracellular fluid in fetuses at various stages of development can be calculated on the assumptions that the placenta does not secrete Na and that the Na^{24} is equally distributed throughout the extracellular fluid and is limited to it. Multiplying the equilibrium values (corrected) at each stage by 100 gives the extracellular fluid weight as per cent of the

body weight. This is 25 per cent for a fetus near term and 60 per cent for a 30 gram fetus.

Rates of placental transfer. The experiments have been planned to measure the rates of placental transfer at different parts of the gestation period. In all experiments measurement has been made of the concentration of Na^{24} in the maternal plasma and of the total quantity of Na^{24} in the fetus (each sample was taken at known interval, about one hour, after intravenous injection into the mother); and of the placental and fetal weights. Such measurements can be expressed in several ways as required to analyze the results from one or another aspect, e.g., in terms of total transfer to a fetus per unit time, of transfer rate per unit weight fetus, or of transfer rate per unit weight placenta. The data necessary for deriving these relations are presented graphically. Figure 6 gives the data on changes in placental weight with fetal weight (fetal weight can be translated into gestation age by reference to fig. 8). The change in total hourly transfer of Na^{24} to the fetus with change of fetal size is presented in figure 7.

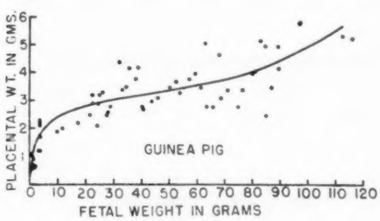

Fig. 6

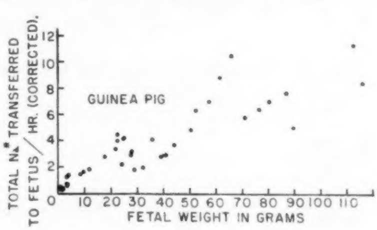

Fig. 7

The data of figures 6 and 7 have been used to construct the curves of figures 8 and 9. In figure 8 are shown the changes in rate of transfer across a unit weight of placenta throughout most of intra-uterine life. As is clear from the curve, the transfer rate per unit weight placenta makes a striking overall increase with age. Thus at 28 days the transfer rate is about 0.2 unit; at 46 days, 1.4 units, and at 62 days, about 1.9 units. Transfer of Na^{24} across a unit weight of the 62-day placenta is consequently about 10 times as fast as across a unit weight of the 28-day placenta. The rise in transfer rate with fetal age does not appear to be continuous, however. An apparent minimum occurs at a gestation age of about 50 days and there is evidence of a decrease as term is neared. The indicated minimum at 50 days is determined by the findings on 3 litters, totaling 9 fetuses.

Figure 9 gives the rate at which Na^{24} is supplied from the maternal plasma to each gram of fetus as this rate varies with fetal age. This rate is high in the early fetus and falls with increase of fetal age. Thus in the 28-day fetus about 0.5 unit Na^{24} is transferred per gram fetus per

hour; in the 40-day fetus, about 0.16 unit and in the 62-day fetus, 0.1 unit. Figure 9 also shows the daily per cent weight increase of the fetus during the greater part of the gestation period (the daily per cent weight increase at any point is 100 times the slope of the tangent to the age-weight curve at that point). This has been calculated from the data of Ibsen as quoted

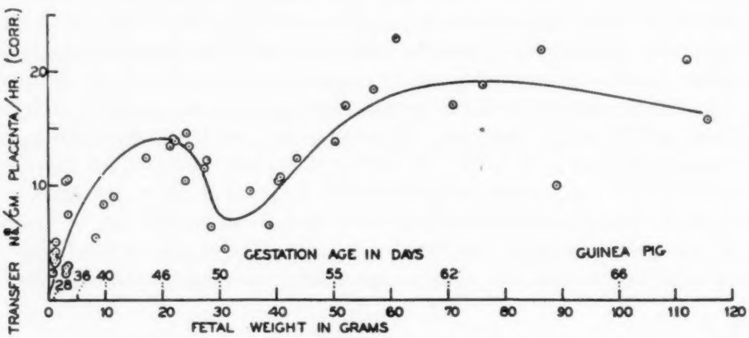

Fig. 8. (The gestation age has been estimated from the data of Ibsen (13).)

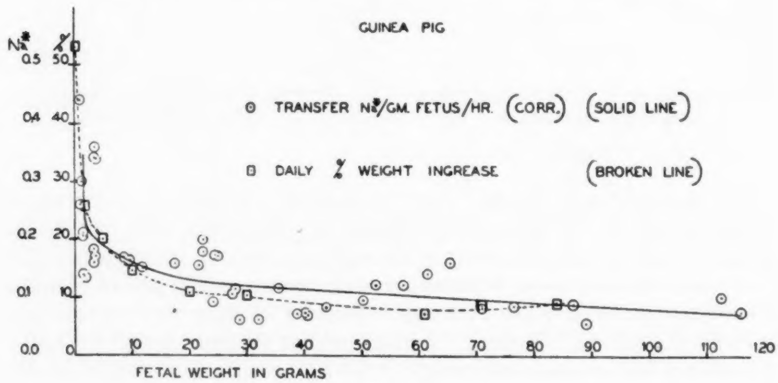

Fig. 9

by Needham (19). It is apparent from figure 9 that the parallelism of these 2 curves is striking.

Fetal need for Na relative to supply across placenta. By using the transfer rate of Na^{24}, the equilibrium concentration of Na^{24} and the hourly per cent growth rate of the fetus, it is possible to calculate by just what factor the supply of Na exceeds the need. The ratio of the supply of Na to the amount of Na retained by the fetus will be designated the safety factor.

The safety factor is readily calculated. The Na^{24} present in the maternal circulation is thoroughly mixed with all the Na of the maternal plasma in a fixed proportion. It may consequently be assumed that any Na^{24} which passes from the mother to the fetus is accompanied by Na from the mother and in the same proportion, provided no separation of isotopes occurs. Hence the Na supplied to a unit weight of fetus per hour is directly proportional to the amount of Na^{24} supplied to it per hour. On the other hand, the hourly accretion of Na by this growing unit of tissue is a direct function of the hourly weight increase of the tissue and of the amount of Na per unit weight of tissue. This last, the amount of Na in a unit weight of tissue, is directly proportional to the concentration of Na^{24} per gram reached by the fetal tissue when at equilibrium with the maternal plasma. The safety factor is then:

Transfer Na^{24} per gram per hour (corr.) × 24 hrs. × 100 ÷ equilibrium concentration Na^{24} per gram (corr.) × daily per cent weight increase. The value of the safety factor for guinea-pig fetuses is given in table 1.

TABLE 1

FETAL WEIGHT	TRANSFER Na^{24} PER GRAM FETUS PER HOUR (CORR.)	DAILY PER CENT WEIGHT INCREASE	EQUIL. CONC. Na^{24} PER GRAM FETUS (CORR.)	SAFETY FACTOR	TOTAL Na TRANSFERRED TO FETUS PER HOUR	TOTAL Na RETAINED IN HOURLY GROWTH OF FETUS
gms.					*mgm.*	*mgm.*
100	0.08	10	0.27	65	26	0.4
30	0.12	10	0.63	50	12	0.25
3	0.20	22	(0.75)	30	2	0.07
1	(0.45)	33	(0.8)	(40)	1.5	0.04

The data of columns 2 and 3 have been interpolated from figure 9; the data of column 4, from figure 5. Values in parentheses have been extrapolated.

The quantity of Na transferred to the fetus per hour as well as that incorporated in new growth can be separately calculated by assuming a reasonable value for the concentration of Na in the maternal plasma. If the total Na and total Na^{24} transported to the fetus per hour be designated respectively by Na_F and Na^{24}_F, and the concentration of Na and Na^{24} in the maternal plasma, by $Na_{M.P.}$ and $Na^{24}_{M.P.}$; then:

$$Na_F = Na^{24}_F \times \frac{Na_{M.P.}}{Na^{24}_{M.P.}}$$

The expression on the right is simply the total Na^{24} transfer per hour (corrected) multiplied by the concentration of Na in the maternal plasma.

The hourly accretion of Na by the growing fetus is: $Na_{M.P.}$ × equilibrium concentration Na^{24} per gram (corr.) × fetal wt. × daily per cent weight increase ÷ 24 × 100.

The quantities of Na transferred per hour and retained in hourly growth are given in columns 6 and 7 of table 1. Their ratio is the safety factor. They have been derived from the data of figure 7, assuming a concentration of 3.3 mgm. Na per cubic centimeter of maternal plasma.

Discussion. In the stages of gestation studied here, an increase of over a hundredfold in fetal weight is accompanied by only about a twelvefold increase in placental weight (fig. 6). The gain in placental weight which accompanies fetal growth gives, however, an entirely misleading concept of the change in rate of placental transfer as measured by Na^{24}. An equally important factor is the change which occurs in transfer rate per unit weight of placenta. The experimental findings indicate that the rate of transfer across a unit weight of placenta increases about 10 times from the early to the late stages of pregnancy in the series. It is to be noted that these values apply only to transfer to the fetus proper and do not include fetal blood in the placenta.

The placenta, then, is adapted by changes in its size and in its unit transfer rate to the size and so to the nutritional requirements of the fetus. How good is this adaptation in the guinea pig as measured by Na^{24}? Perfect adaptation from point of view of fetal growth would mean that at those periods of relatively rapid growth, where there is a large demand for substances out of which to build new tissue, there would be a correspondingly rapid transfer across the placenta. An examination of the relative growth curve of the fetus at different periods of pregnancy shows it to be similar to the curve describing the change of rate of transfer to a unit weight of fetus during the gestation period. The transfer rate per gram fetus is high in early pregnancy, despite the low transfer rate per unit weight placenta, because of the large size of the placenta relative to fetal size. The results suggest the hypothesis, wholly tentative, that the fundamental principle underlying change in placental transfer during the gestation period is that placental transfer to the fetus shall parallel the growth rate of the fetus.

It is impossible to give a quantitative explanation of the change in transfer rate from the maternal circulation to the fetus proper found with change of intrauterine age. The magnitude of total transfer to the fetus will depend upon factors which apply both to the decidua basalis and to the placenta. For a particular substance these factors are: the area of surfaces across which transfer occurs, the rate of blood flow past these surfaces, the nature of the tissues separating the two circulations, and the chemical state of the substance being transferred. The increase in weight of the placenta (fig. 6) may be taken as evidence of a purely qualitative kind that the effective surface area increases on the fetal side as gestation progresses. The weight of the decidua basalis reaches its maximum about two-thirds of the way through pregnancy and then slowly declines (13).

There are no observations known to us on the rate of blood flow through the placenta or decidua basalis of the guinea pig. However, direct observations have been made on the rate of blood flow through the placenta of the sheep (3) and through the maternal portion of the uterus in the pregnant rabbit (2); these at best may be considered no more than suggestive for the guinea pig. The findings on the sheep seem to indicate that the rate of flow in the capillaries of the placenta may double during the last fifth of pregnancy. The degree to which this increase in blood flow will increase transfer rate depends upon its effect upon the concentration gradient between the two circulations. Measurement of the rate of blood flow through the vessels of the pregnant uterus of the rabbit during the last half of pregnancy gives evidence which suggests that during this period the rate of blood flow through the decidua basalis does not change considerably.

Of clearer meaning for interpretation of increase in placental transfer are the changes which occur with intra-uterine age in the tissues separating the maternal and fetal circulations. The placenta of the guinea pig, in the classification of Grosser (10), is of the hemochorial type, i.e., throughout the bulk of the placenta, the tissues separating maternal from fetal circulations are entirely fetal and consist of chorionic epithelium and endothelium of fetal blood vessels. With progress of gestation, however, the chorionic epithelium thins (10, 16) and finally so largely disappears that Mossman (17) has suggested the term hemoendothelial for this type placenta. These histological findings fit the conclusion that increase in total transfer as well as increase in transfer rate per unit weight placenta both depend in part upon the thinning of the membranes placed between the fetal and maternal circulations.

We are grateful to the staff of the Department of Terrestrial Magnetism, Carnegie Institution of Washington, for their constant help and advice which made possible the construction of the ionization chamber-string electrometer circuit. We are much indebted to Dean B. Cowie of the National Cancer Institute for making the sodium bombardments with the Carnegie generator. We also wish to acknowledge the kindness of Prof. William M. Nielsen in placing the facilities of the Duke University Instrument Shop at our disposal.

SUMMARY

1. A simple pressure ionization chamber-string electrometer circuit for measurement of radioactivity is described.

2. *a*. The guinea-pig fetus comes to within 10 per cent of equilibrium with Na^{24} in the maternal plasma in from 5 to 7 hours. This compares strikingly with the extracellular fluid of the mother which comes to within 10 per cent of equilibrium with the plasma in about 5 minutes.

b. Large changes take place in the apparent proportion of fetal extracellular fluid to fetal body weight throughout gestation.

3. Changes in rate of placental transfer of Na^{24} per unit weight placenta have been measured from the twenty-eighth day of pregnancy until term. The transfer rate of Na^{24} increases about 10 times during this period.

4. The relative growth curve of the fetus is parallel to the curve describing the change of rate of transfer of Na^{24} to a unit weight of fetus at different periods of pregnancy.

5. The fetus receives across the placenta, throughout the stages of development studied here, an average of about 50 times as much Na as is incorporated in the growing tissues.

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REFLEX STUDIES AFTER MUSCLE TRANSPLANTATION[1]

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Muscle transplantation, i.e., the translocation of the tendinous insertions of muscles, has been used with a great degree of success on human patients. Although every skeletal muscle cannot be transplanted and then perform its new function in the proper way, many cases are on record where the muscles do behave in the expected manner; to do this may require an extensive period of learning if an antagonistic action is conferred (Legg, 1923). Experiments on the lower animals, however, have given uniformly negative results. Taylor (1935) tried to convert the gastrocnemius of a frog into a flexor of the ankle, but was without success. Sperry (1939) crossed the ankle flexors and extensors in rats and even after 10 months' time could find no evidence of reversion of response in ordinary activities or in special trained performances. Others have transplanted supernumerary limbs or muscles in toads and rats and in each instance activity of the transplanted muscles is "homologous," e.g., is similar to that of the corresponding normal muscles and is thus indicative of an inviolable predetermination of response which in some instances may be harmful to the well-being of the animal.

Similarly the insertion of an eye muscle is often shifted in the human patient so as to make that muscle more effective or less so through changing its mechanical advantage. For example, the superior oblique has been substituted for a paralyzed internal rectus, with both cosmetic improvement and restoration of binocular vision (Wiener, 1928; Peter, 1933). More striking still are the experiments of Marina (1912, 1915), who found complete interchangeability of all the eye muscles of monkeys; within four days after crossing the externus and the internus behind the eyeball, convergence and divergence, nystagmus, voluntary movements and cortically-induced movements were claimed to be normal. Dusser de Barenne and de Kleyn (1928) crossed the internus and externus of rabbits and obtained conflicting results. Where the nystagmus was normal it was found that the retractor bulbi muscle was well developed; in other

[1] This investigation was supported by a grant from the Research Board of the University of California.

rabbits, the nystagmus remained reversed up to a year's time. Neither monkeys nor man, however, possess the retractor bulbi muscle. Olmsted, Margutti and Yanagisawa (1936) believed that the return of normal movement subsequent to crossing of the superior and external recti of cats and dogs was due to a process of central relearning, but unpublished observations by the present authors over a three year period have shown that after complete removal of all the extrinsic muscles of the dog's eye, except the retractor bulbi, there follows a gradual regaining of a limited ability to move the eye in all directions, particularly in those dogs whose retractor bulbi is well-developed.

In order to determine whether any possible reorganization of the central nervous system to meet such altered conditions becomes fixed so that the new responses are truly reflex, we have attempted to convert flexor muscles of the limbs into extensors and vice versa in adult cats and dogs, and the superior oblique into an adductor in the rabbit.

In different cats we have tried the following transplants: fastening the tendon of peroneus longus, a flexor, subperiosteally to the calcaneus a short distance behind the ankle joint; sewing flexor digitorum longus to the tendon of Achilles; sewing the tendon of soleus to the tendon of peroneus longus. In the dog we have fastened either tibialis posticus or peroneus longus—both flexors—to the calcaneus. The majority of these operations were unsuccessful since, although the limb was immobilized in a plaster cast for some days after the operation, the new attachments failed to hold. Five operations, however, were successful, and 27 to 126 days were allowed for readjustment to the new situation. The animals were then decerebrated and the isolated reflex activity of the transplanted muscle was contrasted with originally or newly synergic muscles, or with the original antagonist; comparison was also made with the same muscle on the control leg. Various ipsilateral and contralateral sensory nerves were stimulated electrically to produce the reflex responses.

Without exception the transplanted muscles under the influence of reflex activation behaved as they would have done had transplantation not been performed.

The tracing on the left of figure 1 is the record of the reflex contraction of soleus, an extensor, in a cat in which this muscle had been successfully placed in a flexor position, and of its normal antagonist, tibialis anticus, which should now be its synergist. At the time of sacrifice there was no incoördination in the walking of this animal so that from external appearances it might have been inferred that readjustment of the soleus to its new function had been perfected. However, upon decerebration typical exaggerated tone appeared in soleus in spite of its flexor position, and stimulation of the ipsilateral posterior tibial nerve caused inhibition of this tone. The same reciprocal relation of the transplanted soleus with

tibialis anticus that normally occurs in this pair of muscles is well shown in this record.

The central part of figure 1 shows the reflex responses of a dog's flexor muscle, peroneus longus, after being transposed to an extensor position. There was no increase in its tone on decerebration. Stimulation of the ipsilateral popliteal nerve is here seen to result in a co-contraction with its normal synergist, tibialis anticus, which should now be acting as its antagonist. Stimulation of the contralateral popliteal nerve, instead of causing contraction of the transposed peroneus longus, resulted in inactivity of it and of its normal synergist as shown in the right hand part of figure 1.

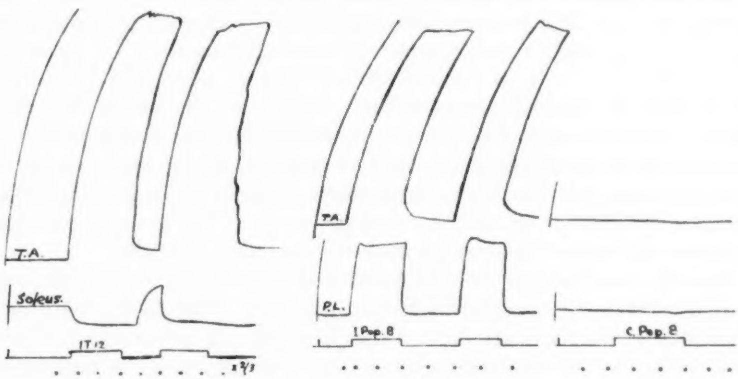

Fig. 1. At left: reflex response of tibialis anticus (flexor) and soleus (extensor) of a cat—the latter muscle transposed to the flexor position—to stimulation of the ipsilateral tibial nerve, showing undisturbed reciprocal innervation. In center: reflex response of tibialis anticus (flexor) and peroneus longus (flexor) of a dog—the latter muscle transposed to an extensor position—to stimulation of the ipsilateral popliteal nerve, showing undisturbed co-contraction. At right: reflex response of tibialis anticus and transposed peroneus longus of a dog to stimulation of the contralateral popliteal nerve, showing inactivity of both muscles.

In preparation for the final experiments on the rabbits, a one-stage preliminary operation for intracranial section of the oculomotor nerve was performed (Hines, 1931). Approximately a month was allowed for convalescence to be sure that paralysis of the internal rectus muscle was complete; it was noted that the nictitating membrane remained protruded after paralytic symptoms had set in and that the internal quadrant of the retractor bulbi muscle could still adduct the eye to a slight extent (cf. Dusser de Barenne and de Kleyn, 1928). This was shown by the definite though small inward movement of the eyeball when the rabbit was rotated in a horizontal plane. The superior oblique was then freed beyond the trochlea and sewed to the eyeball above the insertion of the internus in

such a way that there was no slack when the eyeball was in the primary position. Approximately three months were allowed for readjustment.

Three types of control were used: 1, rabbits in which the third nerve had been cut intracranially and the eye muscles left untouched; 2, others in which the superior oblique was transplanted and the third nerve left undisturbed; 3, entirely normal animals, except one whose skull had been trephined as in preparation for cutting the third nerve.

At the time of the final experiment, the procedure was the same for the control and experimental series: the rabbit was decorticated under ether anesthesia; the superior oblique and external rectus muscles were freed from the eyeball, and tied to silk threads which later could be attached to very light isotonic bell-crank levers. As many as possible of the remaining muscles were completely removed from the orbit by means of a tonsil snare. A cord was tied around the optic nerve and ophthalmic artery and the eyeball was completely removed. The rabbit was then placed on a turn-table which could be rotated in a horizontal plane. A Czermak head holder fixed the head in position; it was necessary to fix tightly a brass tube into the orbit in order to render the orbital fascia immovable; and the thorax and legs were tied down. The levers wrote upon a horizontally-placed kymograph mounted upon the turn-table.

Records were made of the rotational- and after-nystagmus due to rotation in clockwise and counterclockwise directions. With these precautions there was scarcely any artefact, so that markings on the drum were very little influenced by movements other than contraction of the muscles in question, particularly during after-nystagmus. The significant feature was the slope of the lines indicating the direction of the slow and fast components of nystagmus. These movements were found to be similar in every respect to those recorded by de Kleyn (1925) from the corresponding eye muscles of normal rabbits during caloric stimulation. It was noted that although the slope of the lines was the same, the base line might change, indicating a change in tone of both the superior oblique and external rectus, during rotation in addition to purely phasic responses.

Since the performance of the superior oblique exactly paralleled that of the external rectus in all cases, it was evident that in spite of transplantation, there was no remodulation in the central nervous system. This was true even in those rabbits in which the internal rectus was functional and might be supposed to be able to facilitate adjustment of the transposed oblique in assuming the new function.

The fact that transposition of muscles in these experiments had in no wise altered the original reflex pattern of response, together with the difficulty experienced in making the transplanted limb muscles hold in their new positions may be considered as supporting Weiss' (1936) theory of "homologous response." Weiss has described experiments on amphibia

which purport to show that muscles possess some biochemical factor, a "modulus" which "specifies" the activity of the nervous centers by way of the peripheral nerves. The modulus is supposed to have control over the activity of the motoneurones in the simple behavioral pattern of the amphibia. Hence, if a muscle remains connected to the same nerve centers regardless of where it has been transposed, it would have to respond in a predetermined fashion. A corollary of this theory would demand that each different muscle must have a different modulus. An experiment by Sperry (1939) on rats is interpreted in this light. The failure to produce alteration in reflex pattern of response in our experiments argues that there is some mechanism which prevents the development of a response proper to the new conditions.

SUMMARY

When tested by reflex activity the isolated responses of translocated muscles of the hind limb of the adult cat and dog and of the translocated superior oblique of the rabbit show no alteration in function, even when as long as four months is allowed for readjustment.

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THE EFFECTS OF DENERVATION AND OF STIMULATION ON EXCHANGE OF RADIOACTIVE POTASSIUM IN MUSCLE

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The experiments which we wish to report represent an attempt to use radioactive potassium to test the theory that stimulation is accompanied by an increase in muscle permeability. We did find evidence of increased penetration of the labelled potassium but this result, in all probability, was chiefly due to an increased circulation while the effect which is due to muscular contraction *per se* would seem to be relatively small or absent.

Measurements of the permeability of muscle to injected radioactive potassium have shown that the penetration into muscle is complete in rats in about 4 hours (Noonan et al., 1940). During the first one or two hours after injection therefore an excellent opportunity presents itself of studying variations in the speed of penetration which can be caused by various procedures.

Methods. These experiments were performed chiefly on rats anesthetized with urethane. Muscles were stimulated either by applying electrodes directly to the sciatic nerve which was exposed as far centrally as possible or by inserting a needle electrode through the skin in the region of the sciatic notch and stimulating the nerve without further operation. After stimulating for 1 hour, or in control experiments after denervation for a period of an hour, the animal was killed by bleeding and matched muscles from the two hind legs were taken for determination of the radioactivity. For samples we usually used the gastrocnemius group, the tibialis group and sometimes the semimembranosus. Muscles were dissolved in concentrated nitric acid in tared weighing bottles. The volume of the digest was determined by weighing and by an independent determination of the specific gravity. A 3 ml. sample of the digest was put into a Bale ionizing chamber (immersion type) and measured with a Geiger-Müller counter (Bale et al., 1939).

Calculations. The radioactivity (RA) and the potassium radioactivity (KRA) are calculated as follows:

$$\text{RA} = \frac{\text{counts found per kgm. of tissue}}{\text{counts injected per kgm. of body weight}}$$

$$\text{KRA} = \frac{\text{RA} \times 100}{\text{mM potassium per kgm. of tissue}}$$

$$\text{Per cent penetration} = \frac{\text{KRA of tissues} \times 100}{\text{KRA of plasma}}$$

Since the differences in RA between paired muscles in the rats were relatively large compared to the possible differences in potassium content due to stimulation we have not usually gone to the trouble of analyzing for potassium and calculating KRA. Since, however, the potassium content of rat muscle is known to be about 113 mM per kgm. (Fenn and Cobb, 1935), the KRA (previously called "potassium activity", Noonan et al., 1940) may be found approximately by multiplying the RA by $\frac{100}{113}$. In the frog experiments the differences in RA due to stimulation were less marked and we have, therefore, analyzed all the frog muscles for potassium.

Results. The results of 6 different types of experiments on rats are shown in table 1. The figures represent the radio activities (RA) of the muscles. The results show that without a single exception the experimental muscles contained a higher concentration of the radioactive potassium than the control muscles. In rats 1 and 2 the muscles of one side were stimulated while those of the other side were left intact and resting as control. The penetration was on the average 5 times as rapid on the stimulated side. The difference was in the same direction but only twice as large as the control value in rats 3, 4 and 5 where the electrodes were applied directly to the peripheral end of the cut sciatic. Repeating the same experiment with rat 6 but with the control nerve cut and not stimulated did not change the result, the penetration still being about 5 times as rapid on the stimulated side. It seemed possible that simply cutting the nerve might permit sufficient vasodilatation to increase significantly the rate of penetration. In rat 7, therefore, the control nerve was cut while on the experimental side the nerve was stimulated without being cut. Here the penetration was 1.5 times more rapid on the stimulated intact side than on the denervated control side. Thus if the circulation increase is the determining factor it can be concluded that stimulation and the consequent accumulation of metabolic products causes a greater increase in the circulation than mere denervation. That denervation by itself does increase the rate of penetration, presumably by increasing the rate of circulation, is shown in rats 8 and 9 where the experimental nerve was cut while the control nerve was intact, neither side being stimulated. In these experiments denervation alone doubled the rate of penetration. Finally in rats

TABLE 1

Penetration of radioactive K into stimulated and resting rat muscles

PROCEDURE	RAT NUMBER	MUSCLE	RADIO ACTIVITY	
			Exper.	Control
Both nerves intact. Exper. side stimulated by needle electrode inserted through skin	1	g	0.91	0.13
		t	1.03	0.09
		b	0.82	0.11
	2	g	2.36	0.75
		t	2.68	0.36
Average			1.56	0.29
Experimental nerve cut and peripheral end stimulated. Control nerve intact	3	g	1.55	1.07
		t	1.49	1.13
		(r*	0.27	1.18)
	4	g	1.19	0.98
		t	1.41	0.42
	5	g	1.34	0.65
		t	1.58	0.51
Average			1.42	0.78
Both nerves cut. Experimental nerve stimulated	6	g	0.60	0.15
		t	0.69	0.09
		s	0.57	0.14
Experimental nerve intact and stimulated. Control nerve cut	7	g	1.60	1.12
		t	1.88	1.11
		s	1.12	0.69
Experimental nerve cut. Neither nerve stimulated	8	g	1.09	0.25
		t	0.92	0.13
		(r*	0.38	0.32)
	9	g	1.38	0.90
		t	1.44	1.03
		b	0.88	0.43
Average			1.14	0.55
Control nerve cut 4 days previously. Rat exercised for 1 hour after injection of radioactive potassium	10	g	0.76	0.60
		t	1.55	1.17
	11	g	1.17	0.77
		t	1.24	0.89
Average			1.18	0.86

Abbreviations: g = gastrocnemius and soleus, t = tibialis and peroneus, b = biceps femoris, s = semimembranosus, r = rectus femoris.

* Not stimulated or denervated and not included in the averages.

10 and 11 one sciatic nerve of each rat was cut under ether anesthesia. Four days later, without further anesthetic, 2 cc. of a 2.6 per cent solution of radioactive potassium chloride were injected intraperitoneally into each rat. Rat 10 was then made to swim for an hour in a water bath at 30°C. while rat 11 continued the normal exercise of cage life for a similar period. At the end of an hour both rats were killed. On the average the penetration was 1.4 times as fast in the exercised muscles of both rats.

In a few of the experiments of table 1 values were also obtained for the potassium content of the muscles and for the radioactivity and potassium content of the plasma. Thus the potassium radioactivities of both muscles

TABLE 2

Potassium contents and percentage penetration in rat muscles

EXPERIMENT	MUSCLE	POTASSIUM IN mM PER KGM.		PLASMA KRA	TIME AFTER INJECTION	PENETRATION	
		Exper.	Control			Exper.	Control
					hours	*per cent*	*per cent*
3	t	108	113				
	g	115	125				
	(r	119	119)				
4	g	88	108	1.48	1.3	91	56
	t	98	81	1.48		97	36
5	g	70	130	1.56	1.5	122	32
	t	93	128	1.56		109	25
6	g	91	114				
8	g	168	124	2.15	1.0	31	10
	t	120	114	2.15		36	6
	(r	125	116	2.15		14	13)

Experiments correspond to rats of same number in table 1.

and plasma could be calculated, the ratio between them being the per cent penetration of the radioactive potassium into the muscle or the per cent of the muscle potassium which had exchanged with the plasma potassium. The values for the potassium contents in these experiments (table 2) show that (with one exception, 4t) the stimulated muscle contained less potassium (per gram wet weight) and the denervated muscle contained more potassium than the control muscles. This is in agreement with previous results (Fenn and Cobb, 1935; Fenn, 1937).

The percentage penetration shown in the last two columns of table 2 indicates that both denervation (no. 8) and stimulation (nos. 4 and 5) markedly increased the rate of exchange. Indeed in rats 4 and 5 the ex-

change in the stimulated muscles appears to be practically complete in 90 minutes while their control muscles had exchanged only about one-third of their potassium in the same period. In this respect stimulated muscle behaves like diaphragm and heart both of which are continuously active and both of which may show potassium radioactivities greater than the simultaneous values (of KRA) in the plasma 1 or 2 hours after injection.

It seems probable that urethane anesthesia retards slightly the penetration into muscle. The average penetration into the control muscles of table 2 is 27 per cent in 1 hour. In our previous experiments with non-anesthetized rats taking only such exercise as is incident to normal cage life we found an average penetration of 47 per cent in 1 hour in 6 experiments (Noonan, Fenn and Haege, 1941).

Values given for the rectus muscle (in brackets, tables 1 and 2) in two experiments may be regarded as control experiments since this muscle was not stimulated and not denervated by treatment of the sciatic nerve. The potassium contents were not significantly changed by the procedure but in one stimulation experiment (no. 3) the radio activity of the rectus muscle was much diminished on the stimulated side compared to the control side as if the stimulated muscles of the same leg had received an increased blood supply at the expense of the rectus.

These experiments show that an increase in circulation such as may be caused by denervation may double the rate of penetration of radioactive potassium. This finding throws some doubt upon the interpretation of the effects of stimulation. The increased rate of penetration which is observed must be due in part at least to an increase in the circulatory rate. By experiments on rats we have not been able so far to rule out circulatory effects and have no evidence therefore that the muscle cell becomes any more permeable when it is stimulated.

In the hope of excluding complicating circulatory effects we turned to some similar experiments with isolated frog muscles. Pairs of muscles were carefully dissected out and divided in matched sets between two vessels each of about 20 ml. capacity and containing 6 ml. of Ringer's solution. Platinum electrodes were sealed through the walls of each of the vessels, emerging inside under the surface of the Ringer's solution. Thus the muscles of one set could be stimulated at will while remaining immersed in the Ringer's solution. Stimulation at just "maximal" intensity was continued for about 1 hour with a series of twitches delivered at a frequency of about 1 per second. At the end of the stimulation period the muscles of the stimulated side were removed from the solution, gently blotted, weighed and digested in nitric acid. The digest was analyzed for potassium and for radioactivity. The results of 2 experiments in table 3 show the final weight (in per cent of the initial weight), the number of counts, the micromols of K per gram of final weight and the counts per micromol of K (the

latter is similar to KRA but is uncorrected for the amount injected). The KRA of the muscle in per cent of the KRA of the solution is given in the last column. It represents the percentage of the total potassium of the muscle which has exchanged with the radioactive potassium of the solution and is therefore the per cent penetration. Of the 5 pairs of muscles studied 3 show slightly more and 2 show slightly less penetration on the stimulated side. On the average therefore there was no effect which could be attributed to stimulation. This would suggest that the effect observed in the rat muscles was entirely due to the secondary effects of muscular activity upon the circulation.

TABLE 3

Penetration of K into stimulated and resting frog muscles in vitro

Two experiments

MUSCLE	STATE	WEIGHT	POTASSIUM	COUNTS PER GRAM	COUNTS PER μM K	PENETRATION
Experiment 12. Stimulate 1 hour at 66 per minute						
		per cent of initial	*μM per gram*			*per cent*
Sartorius	Stim.	113	71.9	4680	65.1	11.3
	Rest	101	80.6	4330	53.7	9.4
Semitendinosus	Stim.	107	70.2	5200	74.1	12.4
	Rest	99	83.4	6310	75.6	13.2
Solution			3.48	2000	574	
Experiment 13. Stimulate 1½ hours at 60 per minute						
Sartorius	Stim.	98	75.0	1534	20.5	14.0
	Rest	96.5	78.6	1553	19.8	13.5
Semitendinosus	Stim.	111	74.8	1385	18.5	12.7
	Rest	99.5	74.7	1350	18.1	12.4
Peroneus	Stim.	107	71.4	699	9.8	6.7
	Rest	99.5	78.1	816	10.5	7.2
Solution			3.42	500	146.2	

S = stimulated, R = resting. Potassium and counts are calculated per gram of final wet weight. Muscles used were sartorious, semitendinosus, ileofibularis and peroneus.

In considering these experiments of table 3 it may be observed that in every case the stimulated muscles gained water in comparison to the controls. It is probable that part of this increase in weight was due to extracellular water. If this had been corrected for by deducting the appropriate

amounts of potassium and of counts from the totals found in the muscle the counts per micromol of K so calculated for the muscle fibers would have been even less on the stimulated side. In every case the potassium per gram of final weight was less on the stimulated side but this was partly due to the increase in weight on that side. When the potassium is calculated on the basis of the initial weight only 3 of the 5 pairs show a loss of potassium, 1 shows no change and 1 an increase. The counts per micromol of potassium are, of course, the same for both methods of calculation.

Two other experiments with frog muscles are reported in table 4. In the first of these (no. 14) radioactive potassium was injected into the dorsal

TABLE 4

Penetration of K into resting and stimulated frog muscles in situ

MUSCLE	STATE	WEIGHT	POTASSIUM	COUNTS PER GRAM	COUNTS PER μMK	PENETRATION
Experiment 14. With natural circulation. Stimulate 1 hour at 66 per minute						
		mgm.	*μM per gram*			*per cent*
Gastrocnemius	Stim.	991	74.2	451	6.1	9.4
	Rest	860	82.0	455	5.6	8.6
Semimembranosus	Stim.	644	76.0	550	7.2	11.2
	Rest	552	87.2	542	6.2	9.6
Rectus	Stim.	1462	74.8	635	8.5	13.1
	Rest	1343	80.8	640	7.9	12.3
Plasma			7.86	508	64.6	
Experiment 15. Perfused frog. Stimulate 15 minutes at 114 per minute						
All on hind legs	S	3581	58.5	1910	32.7	10.8
	R	2904	72.4	2465	34.0	11.3
Plasma—arterial			3.74	1130	302	
Venous before stim.			3.50	932	266	
Venous after stim.			5.51	890	162	

lymph sac and peritoneum of a frog. Twenty-five minutes later the brain was destroyed by a clamp, one sciatic nerve was exposed in the abdomen by an incision in the back, and was stimulated 66 times per minute for an hour. At the end of this time blood was drawn by syringe (with heparin) from the aorta and samples of plasma and hind leg muscles were taken for counting and for K analysis. The muscles of the stimulated side contained uniformly more water and less potassium per gram of wet weight than their controls and a slightly greater fraction of their K had exchanged with radioactive plasma K. This small difference, however, may have been due to an increase in extracellular fluid with potassium radioactivity equal to that of the plasma. If half of the increase in weight of the stimulated

muscle over the control muscles were extracellular this would approximately explain the increased penetrations observed. This experiment, therefore, offers no evidence of increased permeability of muscle on stimulation. Apparently also there was no increase in circulation on the stimulated side. In general the penetration into frog muscle is slower than in rat muscle.

In experiment 15, table 4, the hind legs of a frog were perfused with 3 per cent acacia Ringer's solution plus 10 per cent of washed beef red cells. The venous perfusate was collected from a cannula in the abdominal vein, the renal portal flow being tied off. The potassium in the solution was radioactive. Stimulation of one sciatic nerve at 114 shocks per minute was begun 20 minutes after the flow started and was continued for 15 minutes, after which the muscles were removed for analysis for K and radio K. Venous samples were collected for analysis for 2 minutes before and for the first 3 minutes after stimulation began. The rates of flow measured for these 2 samples were 2.08 and 2.12 ml. per minute respectively. There was, therefore, no increase in flow in the active muscle unless there was a corresponding decrease in the control muscle. All the muscles on each leg were pooled for analysis. The stimulated muscles were obviously wetter, weighed 1.24 times more than their controls and contained less total K and less radio K. The percentage exchanged was if anything less than on the control side. If correction could be made for the extracellular fluid the calculated penetration on the stimulated side would have been even less. This experiment also suggests, therefore, that in perfused frog muscle there is no increase in permeability due to stimulation.

The analyses of the perfusate are of special interest in this experiment. Figures for the counts per gram show that the muscles removed radioactive K from the perfusate both before and after stimulation. Before stimulation the muscles removed a small amount of total K but after stimulation a large increase in the K content of the perfusate was observed. From these figures it is possible to calculate the amount of radio K exchanged for normal K for comparison with the net transfer. Let C = counts per gram, K = micromols of K per gram and subscripts t, a and v refer to tissue, arterial plasma and venous plasma respectively. Let x = micromols of K per gram which exchange.

$$\text{Then } C_v = C_a + \begin{matrix}\text{counts which}\\ \text{come in}\end{matrix} - \begin{matrix}\text{counts which}\\ \text{go out}\end{matrix}$$

$$= C_a + x\left(\frac{C_t}{K_t} - \frac{C_a}{K_a}\right) - \frac{C_a}{K_a}(K_a - K_v)$$

Now $\dfrac{C_t}{K_t}$ is the KRA of the tissue which is unknown but it must have been

very low because at the end of the experiment it was only 34 as compared to $302 = \frac{C_a}{K_a}$ in the artery. If $\frac{C_t}{K_t}$ increases linearly during the perfusion its value at the time when stimulation began may be estimated at about 5 since only $\frac{1}{7}$ of the total volume perfused had been collected at this time. Considerable variations in this estimate will not change the calculation very much. Before stimulation, therefore, the following values may be substituted

$932 = 1130 + x\,(5 - 302) - 302\,(3.74 - 3.50)$, whence $x = 0.42$ micromol of K exchanged per cubic centimeter of perfusate. A similar calculation after stimulation gives a value of 0.93 μmol exchanged $\left(\text{using } \frac{C_t}{K_t} = 20 \text{ in place of } C_a/K_a \text{ in the last term}\right)$. This apparently indicates an increase in the amount exchanged as a result of stimulation, but the error in the radioactivity is such that even this large difference is not significant. The calculation does show, however, the order of magnitude of the exchange which occurs in one capillary transit in addition to the net transport of K.

Discussion. Only in the experiments with frog muscles in vitro has it been possible to rule out possible increases in penetration due to improvements in the circulation. Since under these conditions no increase in permeability was found as a result of prolonged stimulation it is logical to conclude that in the case of rat muscles contracting in situ the increased penetration which was observed was due entirely to an increase in the capillary bed or to an increased rate of flow. From experiments on rats alone this conclusion is not justified although it can be shown clearly that the rate of penetration can be increased by the vasodilatation which follows immediately after denervation.

Our conclusions relative to frog muscles agree well with those of Dean (1940) who observed that over a period of 1 hour the penetration of radioactive K into frog muscle was increased only from 10.6 per cent to 11.2 per cent by substituting nitrogen for oxygen. Thus the penetration seems to be uninfluenced by rather drastic changes in the treatment.

SUMMARY

1. The rate of penetration of radioactive K into rat muscles in 1 hour is increased as much as 5 fold by stimulation and about 2 fold by denervation.

2. In isolated frog muscles stimulated in Ringer's solutions containing radioactive K no increased penetration could be observed. This conclusion was confirmed by stimulation of frog muscles in the body both with natural circulation intact and during perfusion.

3. The experiments indicate that muscular activity does not increase the permeability to potassium, all the increase observed in rat muscles being attributable to an increase in the circulation.

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THE RESPONSE OF THE ADRENALECTOMIZED DOG TO RENIN AND OTHER PRESSOR AGENTS

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Most investigators are agreed that although a moderate hypertension can be developed in an adrenalectomized dog with kidneys rendered ischemic, it is not maintained unless supportive therapy in the form of salt or cortical extract is given (1–6). Usually, but not always (7), the elevated pressure has been reduced to normal or subnormal levels by the time definite symptoms of adrenal insufficiency have appeared. The pressor effect of the ischemic kidney is presumably through the action of renin, which induces a generalized vasoconstriction without influencing directly the heart rate or blood flow (8–11). It has been reported (12–13) that the blood pressure response to renin is greatly diminished in the adrenalectomized animal, a diminution not correlated with either symptoms of insufficiency or the level of the blood pressure, but only with the time interval after supportive therapy is withdrawn. A comparable reduction in response to tyramine, epinephrine or amphetamine was not observed. A direct interrelationship between the hormone or hormones of the adrenal cortex and the sensitivity to renin is therefore indicated. Our interest in the adrenal cortex and in renin led us to investigate further this possible interrelationship.

Methods. The renin used in these experiments was prepared by the method described previously (14). Two general dosage levels were used. The first was large enough to insure the maximum pressor response of the test animal. The extract used yielded on assay (15) a 40 mm. Hg rise in mean pressure with a dose of 0.1 mgm. per kgm. body weight. A total of about 3 mgm. renin of this potency, in a 10 kgm. dog, will evoke the maximum pressor response (16). In these experiments a dose of 9.5 mgm. was used, which is roughly 3x maximum. The second dosage level employed was sufficient to produce a rise of 30 to 40 mm. Hg in the intact dog.

The responses of adrenalectomized dogs to the lower renin dose were compared to those evoked by epinephrine (adrenalin hydrochloride,

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Parke, Davis and Co.), pitressin (Parke, Davis and Co.), and barium chloride, at dosage levels selected to yield a rise of about 30 mm. Hg in mean pressure.

Sixteen healthy adrenalectomized dogs, table trained for blood pressure recording without anesthesia, were used in these experiments. Six of these were given the overdosage of renin, four the low renin dosage, three, epinephrine and barium chloride, and three, pitressin. The time sequence of the testing was selected to minimize any precipitating influence the response to the pressor agent, *per se*, might have had on the normal course of adrenal insufficiency. For example, with the large renin doses, only one test was made in one cycle, extract being restored immediately after the test. For a complete series of pressor responses at different stages of adrenal insufficiency, each animal therefore went through repeated cycles of insufficiency and return to normal.

TABLE 1

Protocol of an adrenalectomized dog run through several cycles of insufficiency to determine changes in the pressor response to overdosages of renin

DATE	SERUM SODIUM	SERUM CHLORIDE	SERUM POTASSIUM	RED BLOOD CELL VOLUME	HEMOGLOBIN	BLOOD UREA-N	BLOOD PRESSURE	RISE IN PRESSURE	PEAK PRESSURE	REMARKS
	m.eq./l.	*m.eq./l.*	*m.eq./l.*	*per cent*	*grams per cent*	*mgm. per cent*	*mm. Hg*	*mm. Hg*	*mm. Hg*	
3/6							97	70	167	Unilateral
3/10							106	70	176	Unilateral
3/27	140.1	113.8	5.1	36.3	13.5	19.9	96	76	172	Maintenance extract
4/2	133.5	100.6	6.1	41.8	13.1	31.2	68	56	124	Insufficiency
4/8	139.7	112.8	5.0	27.1	10.2	19.4	90	72	162	Normal—on extract
4/15	133.7	107.2	6.4	29.9	13.1	46.9	54	45	99	Insufficiency
4/22	142.7	111.8	5.2	22.0		18.6	95	70	165	Normal—on extract
4/23	141.7	117.9	5.3	22.2	8.4	18.8	95	71	166	Off extract—24 hours
4/27	135.1	108.8	5.8	23.7	8.5	22.3	79	83	162	Insufficiency
5/3	130.3	106.4	8.4	33.1	11.1	86.1	64	50	114	Severe insufficiency

Results. I. *Overdosage of renin* (table 1, fig. 1). Control tests on seven unanesthetized intact dogs with the 9.5 mgm. dose of renin ranged from 57 to 77 mm. Hg, with an average of 70 mm. Hg. This rise is, of course, smaller than the maximum obtainable from similar doses in the anesthetized animal (16). Adrenalectomized dogs on maintenance extract show the same order of pressure response.

The data given in figure 1 show that there was no reduction in the capacity of the vasoconstrictor apparatus to respond fully to large doses of renin, despite the falling blood pressure, until rather late in adrenal

insufficiency. Even when the pressure had fallen to 50 mm. Hg or below, the maximum pressor response to these large doses was never entirely lost.

II. *Low renin dosage* (fig. 2). The overdosages of renin obviously measured only the maximum power of constriction, and afforded no direct index to possible changes in sensitivity. This index was obtained by the use of small, clearly sub-maximal doses of renin, given four adrenalectomized dogs in various stages of insufficiency. Figure 2 shows that there was an immediate reduction in response when cortical extract was

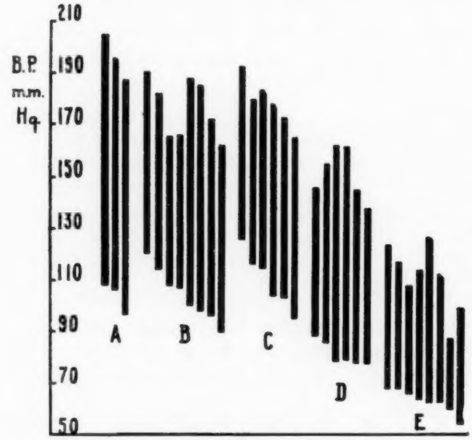

Fig.1

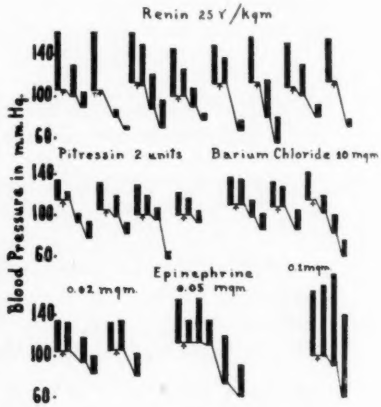

Fig. 2

Fig. 1. Blood pressure responses of adrenalectomized dogs in various stages of adrenal insufficiency to overdosages of renin.

A, unilaterally adrenalectomized, average rise 72 mm. Hg; *B*, bilaterally adrenalectomized, maintenance extract, average rise 72 mm. Hg; *C*, extract withdrawn, no symptoms, average rise 69 mm. Hg; *D*, mild insufficiency, average rise 70 mm. Hg; *E*, moderate to severe insufficiency, average rise 48 mm. Hg.

Fig. 2. Blood pressure responses of adrenalectomized dogs in various stages of adrenal insufficiency to low doses of renin.[2]

↑ Cortical extract withdrawn.

[2] Twenty-five gamma per kilogram body weight yielded an average rise of 35 mm. Hg on assay (15).

withdrawn, even while the arterial pressure and blood electrolyte concentrations were still normal. As symptoms of adrenal insufficiency appeared, the responses to renin became progressively less. When the pressure had fallen to 50 mm. Hg, the average rise was only 27 per cent of the normal.

This decline in sensitivity to small doses of renin was strikingly confirmed by a second series of animals. Four adrenalectomized dogs on minimal maintenance doses of cortical extract showed a distinct tendency toward a response lower than that given by control intact animals (table

2). The injection of large amounts of cortical extract corrected this deficiency. The four dogs were then placed on daily doses of 0.5 mgm. desoxycorticosterone acetate[3] for a period of 14 days. The pressor response was equal to that of the intact animals (table 2).

We have not observed, as did Friedman and co-workers (13), a full response to either large or small doses of renin in an animal with blood pressure at shock levels. Otherwise, our results with the low renin dosage agree with those of these workers and those of Williams and co-workers (12). Since the full response is reduced even on barely maintenance levels of cortical extract, and shows a rapid diminution when extract is withdrawn, it is not surprising that the response is often subnormal in animals receiving only salt therapy (12–13).

TABLE 2

The blood pressure responses to small doses of renin of adrenalectomized dogs on low doses of cortical extract and on maintenance doses of desoxycorticosterone acetate*

INTACT CONTROLS				ADRENALECTOMIZED						
				Cortical extract				Desoxycorticosterone acetate		
Dog	Blood pressure	Peak pressure	Rise in pressure	Dog	Blood pressure	Peak pressure	Rise in pressure	Blood pressure	Peak pressure	Rise in pressure
	mm. Hg	*mm. Hg*	*mm. Hg*		*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*
1	114	150	36	5	114	142	28	122	154	32
2	104	140	36	6	96	122	26	114	153	39
3	116	153	37	7	124	149	25	114	150	36
4	116	145	29	8	92	120	28	128	152	24
Ave.	113	147	34		106	133	27	120	152	32

* One-tenth milligram per kilogram body weight of an extract yielding an average rise of 34 mm. Hg on assay (15).

It has been reported that the loss in pressor response to renin is not true for other pressor drugs, e.g., tyramine, epinephrine and amphetamine (13). Armstrong and co-workers (18) confirmed Elliott (17) that epinephrine in large doses will give full pressor response in terminal adrenal insufficiency, with the blood pressure at shock levels. The results given in figure 2 lend further confirmation, for there was no reduction in response to a dose of 0.1 mgm. epinephrine. Some variation in response to a dose of 0.05 mgm. was observed, with apparently a tendency for the response to diminish as the blood pressure declined. However, evidence of a lessening of response with 0.02 mgm. was seen. We do not know whether the decreased circulation time present in adrenal insufficiency contributed in

[3] We are indebted to the Ciba Pharmaceutical Products, Inc., for generous supplies of the desoxycorticosterone acetate (Percorten) used in these experiments.

any way to this diminution in pressor response to small doses of epinephrine.

The pressor response to splanchnic stimulation, barium chloride, and pitressin are said to be lost in terminal insufficiency (17–18). If this reduction appears early in insufficiency, it would perhaps be comparable to that seen with small doses of renin. Figure 2 shows that the response to pitressin, like that to renin, is often reduced before the arterial pressure has fallen. The sensitivity to barium chloride is lost more slowly, but it is certainly reduced before the arterial pressure has fallen to shock levels. Consistent results with both these drugs were difficult to obtain in the unanesthetized animal, since secondary reactions such as cardiac slowing, vomiting, etc., often were present. No secondary reactions were seen with either large or small doses of renin.

DISCUSSION

The sensitivity to small doses of renin is reduced in the adrenalectomized dog after cortical extract is withdrawn even while the body weight, appetite, blood pressure and level of blood constituents are still entirely normal. In fact, the sensitivity may be partially reduced when merely maintenance doses of cortical extract are given. To a lesser degree, the sensitivity to pitressin is also often reduced. The writers have repeatedly shown that the adrenalectomized dog on maintenance doses of cortical extract shows far less resistance to shock inducing procedures than either the intact animal or the adrenalectomized dog receiving large priming doses of extract. Both lines of evidence seemingly indicate that the vascular peripheral apparatus shows impairment very shortly after the withdrawal of cortical extract, and before detectable symptoms of adrenal insufficiency have appeared. Desoxycorticosterone acetate will seemingly maintain this ability to respond to pressor agents as effectively as will cortical extract, and it will adequately protect the adrenalectomized dog against most types of shock inducing procedures (21).

When definite symptoms of adrenal insufficiency have become manifest, the ability to respond to small doses of renin is but a fraction of the normal. Likewise the pressor response to pitressin, barium chloride, and probably to small doses of epinephrine has been reduced. The underlying factor in this loss in sensitivity of the vascular periphery of the adrenalectomized dog not receiving cortical extract cannot be defined at the present time. It is not simply an absolute loss in power of arteriolar constriction, for there is usually little consistent reduction in response to large doses of epinephrine, even when the animal is moribund. Large doses of renin elicit full pressor response until relatively late in adrenal insufficiency. Although knowledge of the action of renin is incomplete, it is presumably not sympatho-mimetic (19–20). The contrasting failure of pitressin and

barium chloride to stimulate the smooth musculature of the arterioles in terminal adrenal insufficiency (17–18) could conceivably be a reflection of inadequate dosage.

It would seem not entirely valid to assume that the lessening of the pressor response to renin by the adrenalectomized animal not receiving extract indicates a specific interrelationship between renin and the adrenal cortex, any more than the loss in sensitivity to pitressin and barium chloride is indication of a specific relationship. The decreased ability to respond to pressor drugs, including renin, seemingly reflects the dependence of the functional integrity of some part of the vascular peripheral apparatus upon the presence of hormone or hormones of the adrenal cortex. Our experiments indicate that this hormone may be desoxycorticosterone acetate.

SUMMARY

1. The maximal power of vasoconstriction of the adrenalectomized dog, as measured by the response to overdosages of renin, is not reduced until the arterial pressure has fallen to shock levels.

2. The pressor response to small doses of renin is reduced shortly after cortical extract is withdrawn, and before definite changes in blood chemistry or blood pressure have appeared. In severe insufficiency the response has been largely lost.

3. A partial loss in sensitivity to pitressin and barium chloride, and perhaps to small doses of epinephrine, is also present in early insufficiency. There was no consistent reduction to large doses of epinephrine.

4. The loss in sensitivity to small doses of renin does not necessarily indicate a specific interrelationship between the adrenal cortex and renin, but rather reflects a loss of functional integrity of some part of the peripheral vascular apparatus.

5. Adrenalectomized dogs maintained on the synthetic steroid desoxycorticosterone acetate gave full pressor responses to renin.

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THE CALORIE INTAKE AND WEIGHT BALANCE OF HYPERTHYROID DOGS IN RELATION TO VITAMIN B_1 AND YEAST[1]

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It has been shown by many investigators that there is a definite relationship between the food intake of an animal and the requirement for undifferentiated vitamin B. In 1927 Cowgill and Klotz showed that the vitamin B requirement of various species of animals could be approximated by the expression (3):

$$\frac{\text{Vitamins}}{\text{Weight} \times \text{Calories}} = \text{K}$$

A similar relationship was also shown by Plimmer, Rosedale and Raymond (15).

This equation was tested experimentally by Cowgill et al. (5) by increasing the voluntary food intake of the experimental animals by vigorous exercise, and they found that the vitamin B requirement was increased. This relationship was also tested by increasing the metabolism by the feeding of thyroid gland (4, 10). For example, Himwich et al. (10) found that anorexia was produced in normal dogs, with a yeast free diet, in an average of 34 days, whereas thyroid fed dogs on the same diet showed a loss of appetite in 17 days. During this time the thyroid fed dogs ate twice as many calories as the control dogs. This clearly shows that the amount of vitamin B required by an animal increases in proportion to the amount of food metabolized.

Sure and Buchanan (17) have shown that crystalline vitamin B_1 will partially protect thyroid fed rats against a loss of weight. Drill and Sherwood (7) demonstrated that thyroid fed rats which had lost weight, would regain their lost weight when both vitamin B_1 and yeast were administered, even while the thyroid feeding was continued. Vitamin B_1 supplements alone would stop the loss of weight, but the thyroid fed rats

[1] This work was done in the laboratory of Dr. W. W. Swingle and I am indebted to him for the necessary facilities to undertake this work.

did not gain weight until the other B vitamins were administered. In this paper the food intake and weight balance of thyroid fed dogs has been studied in relation to the yeast and vitamin B_1 content of the diet, the thyroid gland being fed for a long period of time (60–90 days).

METHODS. The dogs used were full-grown males weighing between 9 and 17 kgm. They were fed a stock diet of Purina checkers ad libitum before the experiment was started. Two weeks before the thyroid feeding was begun the dogs were placed on a modified form of Cowgill's casein diet no. III (1), which was then continued throughout the experiment. In the modified diet 21 per cent of lard was used and the butter was replaced by 4 per cent of cod liver oil (U.S.P.), so that a known amount of vitamins A and D was added to the diet. The dogs were allowed to eat as much as they wanted for a three hour period each day. Water was constantly supplied. Each dog also received a daily supplement of yeast no. 17800

TABLE 1

The initial weight, surface area and supplements fed to each dog

DOG NUMBER	THYROID GLAND FED PER KGM. OF BODY WEIGHT	GRAMS OF YEAST FED PER DAY	INITIAL WEIGHT	SURFACE AREA
			grams	*sq. m.*
1	None	2.0	10,720	0.518
2	None	3.1	16,220	0.674
3	0.4	2.2	11,975	0.575
4	0.4	3.2	16,810	0.730
5	0.4	2.7	14,290	0.629
6	0.4	2.6	13,840	0.651
7	0.6	2.6	13,610	0.676
8	0.6	1.7	8,900	0.493

in the proportion of 2 International Units of vitamin B_1 per pound of body weight. The yeast used was a dried baker's yeast containing 23 I.U. of vitamin B_1 per gram and 20 Sherman-Bourquin units of vitamin G (flavin) per gram. This makes the diet normal in all respects and supplies a constant amount of the B vitamins. The thyroid gland fed was Lilly's desiccated thyroid gland (no. 957625) containing 0.213 per cent iodine. The dogs received 0.4 or 0.6 gram of thyroid per kilogram of body weight (table 1).[2]

The surface area of the dogs was calculated, using the formula of Cowgill and Drabkin (2). The surface area of the dogs at the start of the experiment is listed in table 1. If the animals' weight changed to any great extent during the experiment the surface area was recalculated.

[2] The author wishes to thank Dr. H. W. Rhodehamel of Eli Lilly and Co. for supplying the large amount of thyroid gland that was required and Dr. C. N. Frey of the Fleischmann Laboratories for supplying the necessary analysed yeast.

Results. The food intake was calculated as calories consumed per square meter of body surface per hour for each dog. The two control dogs in this experiment, receiving the modified Cowgill diet and yeast supplement, ate an average of 58.2 calories per square meter per hour.

When thyroid feeding was started a marked increase in appetite was obtained, reaching a peak between the third and fourth weeks (fig. 1). At this time the hyperthyroid dogs were eating an average of 97 calories per square meter per hour, nearly twice that of the controls. As the thyroid feeding was continued, however, the food intake began to decline. This drop in food intake is similar to that reported for thyroid fed rats (7),

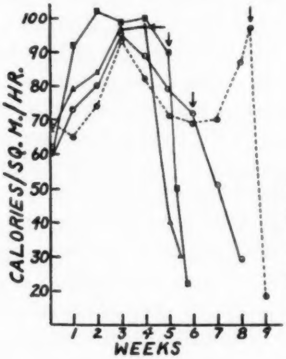

Fig. 1

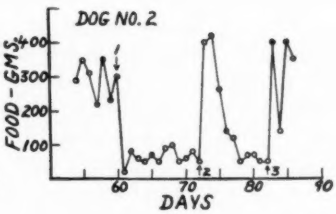

Fig. 2

Fig. 1. The calorie intake per square meter of body surface per hour of the hyperthyroid dogs. Each point is an average value of the calorie intake for the number of days following the preceding point on the graph. Note the marked increase in calorie intake between the second and fourth weeks of thyroid feeding. The arrow indicates the removal of yeast from the diet, which is followed by the marked and sudden drop in appetite.

Fig. 2. The effect of vitamin B_1 on the food intake of a hyperthyroid dog. *1.* Yeast removed from the diet. *2.* An injection of 2 mgm. of crystalline vitamin B_1. *3.* A second injection of 2 mgm. of crystalline vitamin B_1.

and is indicative of diminished body stores of vitamin B_1. It has been reported by Drill (6) and confirmed by Peters and Rossiter (14) that thyroid feeding decreases the amount of vitamin B_1 in rat tissues.

The yeast was then removed from the diet of the hyperthyroid dogs and every dog except no. 6 showed an immediate drop in appetite to a very low level (fig. 1). Some of these dogs even showed a complete anorexia within a few days. Dog 6 had gained weight after the experiment had started, and was thus receiving less than 0.4 gram of thyroid gland per kilogram of body weight. Consequently he had not been depleted of his vitamin stores to any great extent, and his loss of appetite was therefore slower

after the yeast was removed from the diet. Dog 8, receiving 0.6 gram of thyroid gland per kilogram of body weight, spontaneously showed anorexia before the yeast was removed from the diet. Figure 1 clearly shows that the body stores of the B vitamins were depleted, for if the body stores were normal it would have taken 17 days to produce anorexia when the yeast was removed from the diet of the thyroid fed dogs (11).

Weight changes. The two control dogs, fed the modified Cowgill diet plus a daily supplement of yeast, made a slight gain in weight. None of the dogs fed thyroid gland, except no. 8, lost any weight until the yeast was removed from the diet. Dog 8, as mentioned before, was beginning to show signs of anorexia and had therefore lost some weight. As soon as the yeast was removed from the diet of the other hyperthyroid dogs an immediate loss of weight was obtained. The loss of weight always followed the loss of appetite and never preceded it. Dog 6, as mentioned above, showed a slower loss of appetite. The drop in weight of this dog was also correspondingly slower. Thus the change in the food intake, and secondarily the weight of the animal, are not primary effects of thyroid feeding, but are secondary effects due to a deficiency of the B vitamins.

Vitamin B_1 injections. The thyroid fed dogs were then maintained on the modified Cowgill diet for 10 to 15 days, without yeast in the diet. During this period they lost from 0.75 to 1.5 kgm. in weight. Each dog was then injected subcutaneously with 2 mgm. of crystalline vitamin B_1.[3] Within 24 to 48 hours the food intake was stimulated and rose high above normal to the hyperthyroid level (fig. 2). The dog in figure 2 is representative of the other hyperthyroid dogs. The control dogs ate approximately 187 grams of food per day. Dog 2 ate an average of 300 grams of food per day until the yeast was removed from his diet on the 60th day of the experiment, as shown in figure 2. The food intake then dropped below normal and remained low until vitamin B_1 was injected on the 72nd day. The food intake then remained high until the vitamin B_1 was metabolized and/or excreted. On the 82nd day a second injection of 2 mgm. of vitamin B_1 was given and the food intake again increased above normal to the hyperthyroid level. This clearly shows that the high food intake of the hyperthyroid dog is dependent on a normal supply of vitamin B_1, and that when the vitamin B_1 drops below the animals' requirements a rapid drop in appetite ensues, which in turn is followed by a loss of weight.

Discussion. Kunde (11) and McDonald et al. (12) have made various studies on hyperthyroid dogs. They noticed that some of their dogs would gain weight, some would lose weight, and that others would show no change in weight. Kunde stated that her results indicated that neither

[3] The crystalline vitamin B_1 was kindly supplied by Dr. R. T. Major of Merck and Co. as Betabion (Thiamin chloride).

the amount of thyroid fed nor the height of the basal metabolic rate of the dogs determined the percentage loss of weight. Their dogs were fed commercial diets, of which the vitamin potency was not known. In such diets the vitamin intake varies directly with the food intake. Their results are undoubtedly due to a variable food intake with a consequent different vitamin intake. Earlier work on rats, and the present work on dogs, shows the importance of feeding synthetic diets containing a known amount of yeast in all cases where hyperthyroidism is being studied.

In this experiment each dog received a daily supplement of yeast, in proportion to body weight, which is sufficient for normal maintenance. However, if the requirement for the B vitamins is increased the dogs will soon show symptoms of a vitamin B_1 deficiency. Thus the feeding of thyroid gland gave an initial increase in appetite, but as the dietary yeast remained constant the calorie intake soon began to drop. It has already been shown that thyroid feeding will decrease the vitamin B_1 stores of thyroid fed rats (6, 14). The removal of the yeast from the diet at this point causes a rapid decrease in appetite to subnormal levels, some animals showing complete anorexia. With the loss of appetite a decrease in body weight occurs. After the yeast was removed for a few days, and the appetite and weight had fallen, the animal was injected with 2 mgm. of crystalline vitamin B_1 and within 48 hours the appetite had risen to the previous hyperthyroid level. An increase in weight was also obtained. After a few days the vitamin B_1 was excreted and the appetite again fell to subnormal levels, from which it could be raised by injecting vitamin B_1 again.

The experimental work suggests that the continued anorexia and loss of weight in Graves' disease, after iodine therapy, is due to a deficiency of vitamin B_1. Harris and Leong (9) studied the excretion of vitamin B_1 in the urine of humans and concluded that the amount of this vitamin stored in the body was quite small, and that the maintenance of the vitamin stores was dependent on an adequate intake in the diet. Schneider and Burger (16), using the method of Jansen, reported that control patients excreted 80 to 100 gamma of vitamin B_1 per day in the urine, whereas patients with Graves' disease excreted an average 224.8 gamma of vitamin B_1 per day. After operation the vitamin B_1 excretion fell to a normal average of 79.9 gamma per day.

Means et al. (13) have mentioned that yeast increased the appetite and weight of a small series of thyrotoxic patients. Frazier and Ravdin (8) studied the effect of vitamin B_1 on the preoperative preparation of the hyperthyroid patient. They found that 72 per cent of the vitamin treated group gained weight, whereas only 28.5 per cent of the control group gained weight. The vitamin treated group showed a greater increase in appetite than the control group. This is the improvement that would be expected

if some degree of vitamin B_1 deficiency existed. The greatest improvement in the vitamin treated group was observed in the more toxic patients, which is the group in which a vitamin deficiency is more likely to develop.

The evidence presented shows that a deficiency of vitamin B_1 can occur in hyperthyroid dogs *which contain a minimal but normal amount of yeast in the diet.* This deficiency occurs before the weight drops. When the appetite had begun to decline, the removal of the yeast from the diet caused anorexia within a few days, whereas in an animal with normal body stores of vitamin B_1 the removal of the dietary yeast resulted in anorexia in 34 days. In human patients with moderate or severe hyperthyroidism the vitamin B_1 requirement may also be increased sufficiently to produce a loss of weight and anorexia.

SUMMARY AND CONCLUSIONS

1. The food intake of 6 dogs, receiving a normal diet containing yeast, increased to nearly twice the normal level when thyroid feeding was started. After 3 to 4 weeks of thyroid feeding the food intake began to decline, indicating depletion of the body stores of the B vitamins.
2. When the yeast was removed from the diet of the hyperthyroid dogs the appetites rapidly fell below normal, complete anorexia being produced in some cases. The subcutaneous injection of 2 mgm. of crystalline vitamin B_1 restored the appetite to the previous hyperthyroid level within 24 to 48 hours.
3. The decrease in appetite of the thyroid fed dogs is due to a deficiency of vitamin B_1. Anorexia is not a primary effect of hyperthyroidism but is a secondary symptom due to a deficiency of vitamin B_1.
4. The weight of the hyperthyroid animals was maintained as long as no deficiency of the B vitamins occurred. As soon as the yeast was removed from the diet a drop in food intake took place which was followed by a secondary loss of weight.
5. The previous variability of the weight of hyperthyroid dogs reported by other authors is probably due to the difference in the intake of the B vitamins in the diet of the animals.

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THE EFFECT OF THIAMIN ON THE INTESTINE OF THE B_1-DEFICIENT RAT

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It has frequently been observed that crude vitamin B products increase intestinal motility of deficient animals. Observations made after the identification of thiamin as the anti-neuritic principle suggest that vitamin B_1 is the factor responsible for the effect. Indeed, Agid, Beauvallet and Minz (1) have shown that the sensitivity to acetylcholine of the isolated rat intestine is increased in the presence of thiamin and this was found to be true for the intestine of the B-deficient pigeon (2). From these considerations it appears that it might be possible to demonstrate an effect of thiamin on the intestines of normal as compared with B_1-deficient animals and this investigation was undertaken to determine whether an immediately demonstrable effect could be established.

EXPERIMENTAL. The apparatus used was essentially that of Polansky (3). Slight changes in our apparatus made easier the substitution of successive strips of ileum without changing the physical principles involved. An excellent account of these is given by Polansky. With the apparatus any desired liquid can be passed through the lumen of a strip of intestine at controlled rates and pressures. The recording of longitudinal and circular contractions and the maintenance of a constant temperature are provided for.

The rats, all approximately 3 months old, were fed the following diet until a definite weight loss was observed: casein 20; sugar 52; powdered agar 3; salts 5; autoclaved yeast 20; two drops of cod liver oil per day. The controls received in addition 1 mgm. of thiamin per day. All animals were therefore fed on a diet of similar physical properties to avoid changes in motility caused by such a difference in the food eaten.

Since preliminary observations showed that urethane injected intraperitoneally into the rat had no effect on the motility of the ileum as already shown by Alvarez and Hosoi (4), some of the animals were anesthetized with urethane while others were decapitated. Anesthetization with urethane permits the use of many segments of intestine from the same animal. After the rat, which had previously been starved for 24 hours, was

killed or anesthetized, the abdominal cavity was opened, a strip of intestine 5 to 7 cm. long removed from the distal end of the ileum and the abdominal cavity covered until another strip was needed.

The segment of intestine was attached to the apparatus and warm (37°C.) Ringer's solution passed both outside the intestine and through the lumen while the contractions were recorded continuously. If desired, then, the fluid passing through the lumen was changed to thiamin chloride ($1:10^6$) in Ringer's. It is well to emphasize that the pressure within the strip of intestine was unchanged when the perfusing fluid was changed; if this were not so, changes in motility might have been the result of factors other than the addition of thiamin.

For the intestine of normal animals the preliminary experiments indicated that the addition of thiamin to the perfusing fluid had no effect demonstrable by this method, which was now applied to the B_1-deficient

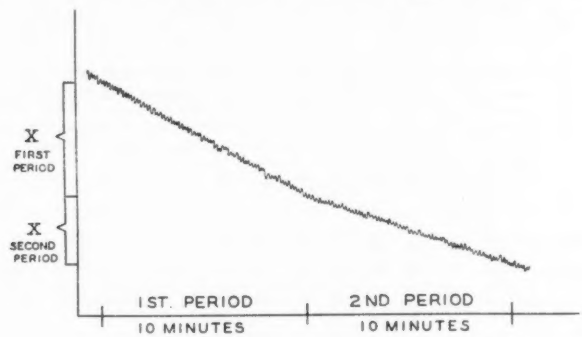

Fig. 1. Showing measurement of tonus loss

rats. Records of activity of 27 strips of intestines from deficient animals were taken with the addition of thiamin to the perfusing fluid after an initial period of perfusion with Ringer's. Likewise records of 15 strips were made without the addition of thiamin but perfused with Ringer's solution throughout.

The amplitudes of contraction were measured and recorded for successive ten minute periods before and after the addition of thiamin and for an equal interval without thiamin. From the beginning of the experiment the strip of intestine gradually lost tonus as indicated by a steady fall in the pointer registering contractions. The vertical distance through which the lever fell in unit time is a measure of the rate of loss of tonus and is so recorded in the table. Figure 1 illustrates the type of curve and the readings recorded.

Results. The table (table 1) shows that for deficient animals the rate of loss of tonus is retarded after the addition of thiamin while the ampli-

TABLE 1

ANIMALS ON ADEQUATE DIET											
Rate of loss of tonus, mm./10 min.						Amplitude of contraction, mm.					
Thiamin added at end of 1st period			No thiamin added			Thiamin added at end of 1st period			No thiamin added		
Period 1	Period 2	Diff.	Period 1	Period 2	Diff.	Period 1	Period 2	Diff.	Period 1	Period 2	Diff.
1.2	0.7	−0.5	0.6	0.6	0.0	0.40	0.40	0.00	0.30	0.20	−0.10
1.3	1.0	−0.3	0.5	0.3	−0.2	0.30	0.10	−0.20	0.90	0.90	0.00
1.1	1.0	−0.1	0.5	0.3	−0.2	1.20	0.90	−0.30	1.70	1.80	0.10
1.4	1.5	+0.1	1.2	0.5	−0.7	1.10	1.10	0.00	0.50	0.50	0.00
1.3	1.2	−0.1	0.4	0.6	+0.2	0.90	0.90	0.00	0.30	0.20	−0.10
0.8	0.5	−0.3	0.1	0.1	0.0	0.70	0.60	−0.10	0.10	0.10	0.00
1.2	1.0	−0.2	1.0	0.7	−0.3	0.10	0.03	−0.07	0.20	0.20	0.00
1.0	1.0	0.0	1.2	0.9	−0.3	1.20	1.20	0.00	0.20	0.20	0.00
1.0	0.7	−0.3	1.2	0.6	−0.4	1.10	1.10	0.00	1.50	1.50	0.00
1.1	1.1	0.0				0.80	0.80	0.0			
$\bar{x}_1 = -0.17$, $\sigma^2 = 0.03344$			$\bar{x}_2 = 0.2\bar{1}$, $\sigma^2 = 0.06861$			$\bar{y}_1 = -0.067$, $\sigma^2 = 0.011$			$\bar{y}_2 = 0.02\bar{2}$, $\sigma^2 = 0.0030$		
$t_{x_1x_2} = 0.4$ $(P > .99)$						$t_{y_1y_2} = 1.3$ $(P > .9)$					

ANIMALS ON B_1-DEFICIENT DIET											
Rate of loss of tonus, mm./10 min.						Amplitude of contraction, mm.					
Thiamin added at end of 1st period			No thiamin added			Thiamin added at end of 1st period			No thiamin added		
Period 1	Period 2	Diff.	Period 1	Period 2	Diff.	Period 1	Period 2	Diff.	Period 1	Period 2	Diff.
1.7	0.5	−1.2	0.1	1.8	+1.7	0.20	0.30	0.10	0.27	0.10	−0.17
2.7	1.2	−1.5				0.00	0.40	0.40			
1.8	0.0	−1.8	0.3	1.6	+1.3	0.10	0.60	0.50	0.30	0.50	0.20
1.6	0.9	−0.7				0.05	0.05	0.00			
0.6	0.2	−0.4	1.6	1.3	−0.3	0.00	0.30	0.30	0.80	0.40	−0.40
1.6	0.5	−1.1				0.50	0.53	0.03			
1.8	0.5	−1.3	4.0	1.8	−2.2	0.40	0.55	0.15	0.40	0.20	−0.20
3.8	1.0	−2.8				0.15	0.60	0.45			
1.6	0.4	−1.2	2.0	1.6	−0.4	0.20	0.30	0.10	0.40	0.40	0.00
0.5	0.2	−0.3				0.09	0.16	0.07			
3.0	1.5	−1.5	0.2	0.8	+0.6	0.10	0.50	0.40	0.15	0.20	0.05
1.0	0.4	−0.6				0.10	0.15	0.05			
0.9	0.5	−0.4	0.8	0.5	−0.3	0.70	0.70	0.00	0.20	0.06	−0.14
1.5	0.1	−1.4				0.03	0.03	0.00			
2.0	0.7	−1.3	3.0	3.0	0.0	0.40	0.40	0.00	0.06	0.09	0.03
1.0	1.3	+0.3				0.05	0.10	0.05			
1.6	0.8	−0.8	0.9	1.7	+0.8	0.04	0.04	0.00	0.05	0.10	0.05
2.5	1.9	−0.6				0.04	0.04	0.00			
2.4	0.4	−2.0	1.7	0.9	−0.8	0.25	0.10	−0.15	0.30	0.20	−0.10
0.8	0.3	−0.5				0.10	0.20	0.10			
2.7	1.3	−1.4	0.9	1.5	+0.6	0.05	0.30	0.25	0.60	0.70	0.10
2.7	0.8	−1.9				0.24	0.24	0.00			
1.4	0.7	−0.7	0.5	2.0	+1.5	0.04	0.08	0.04	0.06	0.03	−0.03
1.2	0.5	−0.7				0.02	0.05	0.03			
2.6	0.9	−1.7	3.9	1.7	−2.2	0.14	0.21	0.07	0.01	0.06	0.05
1.2	0.9	−0.3				0.00	0.17	0.17			
1.3	0.6	−0.7	6.0	3.5	−2.5	0.04	0.10	0.06	0.10	0.05	−0.05
			1.3	1.3	0.0				0.40	0.20	−0.20
$\bar{x}_1 = -1.05$, $\sigma^2 = 0.44423$			$\bar{x}_2 = -0.14\bar{6}$, $\sigma^2 = 1.76981$			$\bar{y}_1 = 0.117$, $\sigma^2 = 0.02609$			$\bar{y}_2 = -0.054$, $\sigma^2 = 0.02261$		
$t_{x_1x_2} = 2.85$ $(P < .01)$						$t_{y_1y_2} = 3.28$ $(P < .01)$					

tude of contraction is increased. The variation among the normal as well as the deficient animals is considerable but the differences in the motilities under the two conditions are apparent. These are confirmed by statistical analysis where the t-values show significant differences for the deficient but not for the normal animals.

The results agree with the work of Abderhalden and Abderhalden (5) recently extended by Briem (6) which showed that thiamin increases the action of acetylcholine. This in turn is explained by the work of Glick and Antopol (7) who showed that the hydrolysis of acetylcholine was slowed down in the presence of thiamin because of the greater affinity of the esterase for thiamin as compared with acetylcholine.

Thus thiamin in concentrations close to those that might be found in the body does act at once to increase the activity of the intestines of vitamin B_1-deficient rats. While this does not necessarily assert that the total effect of thiamin in improving bowel action is only directly upon the gut, it is clear that a direct effect is important. The integration of many such small increases as observed here *in vitro* applied along the whole length of the gut could conceivably become very effective.

SUMMARY

Thiamin chloride perfused through the isolated intestine of a normal rat has no effect within the limits of the method of observation used in these experiments. On the other hand, the peristalsis of the intestines of a B_1-deficient rat is increased and the rate of tonus loss retarded within ten minutes after the addition of thiamin.

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CEREBRAL BLOOD FLOW AND BRAIN METABOLISM DURING INSULIN HYPOGLYCEMIA[1]

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Sakel's treatment for schizophrenia has afforded an opportunity to study brain metabolism of the human during hypoglycemia. A decrease in the cerebral arterio-venous oxygen difference has been reported (1) (2). This was interpreted to indicate a decrease of brain metabolism because Loman and Myerson (3) have demonstrated a fall of blood flow through the human brain during insulin hypoglycemia, and Leibel and Hall (4) found no significant change in the cerebral blood flow of rabbits subjected to insulin coma unless convulsions occurred. Abramson et al. (5), however, observed an increased peripheral blood flow through the extremities during the insulin treatment. Though their data yield no evidence on cerebral blood flow, these workers nevertheless suggested that the smaller arterio-venous oxygen difference during insulin hypoglycemia was caused by a faster blood flow. It is true that conclusive information regarding changes of brain metabolism could be gained only by determining simultaneously both cerebral blood flow and arterio-venous oxygen difference. For this reason arterio-venous oxygen differences and cerebral blood flow were determined in patients with schizophrenia. The effects of various substrates on brain metabolism during hypoglycemia were also observed.

METHOD. Methods for the collection and analysis of the blood samples have been previously described (2). In addition pyruvic acid (6) and bisulfite binding substances (7) were determined.[2] The rate of blood flow in the internal jugular vein was estimated by a modification of the Gibbs thermostromuhr. This instrument proved to be at least as sensitive as the original and has an error of ± 10 per cent for a single reading. For that reason repeated readings were made to determine

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[2] We wish to acknowledge the coöperation of Dr. Ernst Bueding for the determination of pyruvic acid and the bisulfite binding substances.

TABLE 1

Effect of glucose on arteriovenous oxygen differences and cerebral blood flow during insulin hypoglycemia

1	2	3	4	5	6	7
PATIENT AND DATE	TIME	OXYGEN DIFF.	GLUCOSE ART.	PER CENT CHANGE BLOOD FLOW		REMARKS
				Observed	Calculated	
				per cent	*per cent*	
Br	8:10	6.14	83			110 units insulin 8:40
6/21/40	10:40	4.07	19	−10	+51	
	11:40	2.16	15	−30	+184	
	12:40	2.33	17	−10	+168	
	1:40	1.34	13	0	+358	
C	8:50	6.84	89			425 units insulin 9:15
6/22/40	10:15	7.53	65			
	11:15	6.95	43	−10	+4	
	12:15	6.25	33	−10	+15	
	1:15	5.46	24	+10	+32	
	2:15	3.72	32	−20	+94	
T	9:30	6.58	101			140 units insulin 10:00; 25 gm. glucose 1:55; 1:58 aroused
6/24/40	11:50	3.14	35	0	+104	
	1:45	3.55	29	0	+85	
	2:35	6.46	122	−30	+2	
C	9:30	6.69	97			500 units insulin 9:37; 25 gm. glucose 2:39; 2:43 aroused
7/1/40	1:25	4.59	43	+10	+46	
	2:30	4.17	39	+20	+60	
	3:33	5.70	22		+17	
Br	10:08	6.57	94			125 units insulin 10:16; 25 gm. glucose 1:42
7/5/40	12:06	0.62	23	−20	+960	
	1:33	2.45	22	−30	+168	
	2:06	6.62		0	−1	
Br	10:54	1.54	34			25 gm. glucose 10:56; 10:59 aroused
6/27/40	11:15	5.32	117	−20	−246	
B	12:04	3.66	38			25 gm. glucose 12:18; 12:22 aroused
6/27/40	12:36	6.10	139	−10	−67	
W	11:01	4.16	53			25 gm. glucose 11:16
6/29/40	11:28	6.08	127	+10	−46	

blood flow at any given moment. Observations on the blood flow were made over periods up to six hours during which time the position of the thermostromuhr needle, in respect to the vein, was not changed. A special

technique was devised which prevented error resulting from the formation of clots on the needle and served to detect any change in tissue blood flow which may have taken place during the experiment. This method will be described in detail elsewhere. The patients were studied before the injection of insulin and during coma when glucose, sodium lactate or sodium pyruvate was injected intravenously.

TABLE 2

Effect of insulin hypoglycemia on brain metabolism

1	2	3	4	5
PATIENT AND DATE	BLOOD FLOW RATIO	ARTERIO-VENOUS DIFFERENCE VOLUMES PER CENT		BRAIN METABOLISM
		Observed	Corrected	
				per cent
Br	1.00	6.14	6.14	100
6/21/40	0.90	4.07	3.66	60
	0.70	2.16	1.51	25
	0.90	2.33	2.09	34
	1.00	1.34	1.34	22
C	1.00	7.18	7.18	100
6/22/40	0.90	6.95	6.29	88
	0.90	6.25	5.63	78
	1.10	5.46	6.01	84
	0.80	3.72	2.98	41
T	1.00	6.58	6.58	100
6/24/40	1.00	3.14	3.14	48
	1.00	3.55	3.55	54
	0.70	6.46	4.72	72
C	1.00	6.69	6.69	100
7/1/40	1.10	4.59	5.05	75
	1.20	6.17	5.00	75
	1.00	5.70	5.70	85
Br	1.00	6.57	6.57	100
7/5/40	0.80	0.62	0.50	8
	0.70	2.45	1.72	26
	1.00	6.62	6.62	101

Results. Table 1 presents a summary of the observations of cerebral blood flow and arterio-venous oxygen difference before and after the injection of insulin as well as those changes caused by the administration of glucose during hypoglycemia. In 5 cases blood flow and arterio-venous oxygen differences were determined before the injection of insulin as well as throughout the course of the coma and the subsequent administration of

glucose. In 3 other instances observations were begun during coma and continued until after arousal with intravenous glucose. In column 6 are presented the calculated changes in blood flow necessary to cause the changes of the cerebral arterio-venous differences if brain metabolism remained unchanged.[3] Column 5 contains the observed changes in blood flow. In none of these experiments could the low arterio-venous oxygen difference during hypoglycemia be accounted for by any change in blood flow, and in most cases the changes in blood flow were opposite in direction, showing a slow blood flow with a low arterio-venous difference. The average change of 14 observations made during hypoglycemia is −7 per cent.

TABLE 3

Effect of lactate and pyruvate on arterio-venous oxygen differences during insulin hypoglycemia

PATIENT AND DATE	TIME	OXYGEN DIFF.	GLUCOSE		LACTIC ACID		PYRUVIC ACID		REMARKS
			Art.	Ven.	Art.	Ven.	Art.	B.B.S.	
Br	12:30	1.63	17.0	12	13.0	10			20 gm. r-sodium
7/8/40	12:44	1.93	17.0	14	40.0	43			lactate 12:35
T	11:40	4.64	20.0	10	16.0	12			20 gm. r-sodium
7/8/40	11:56	3.58	19.0	8	24.0	23			lactate 11:44
W	11:25	2.65	19.0	15	10.0	8			20 gm. r-sodium
7/9/40	11:40	2.58	13.0	13	25.0	27			lactate 11:32
Br	11:30	2.13	19.0	24	14.0		1.50		7.5 gm. sodium
7/10/40	11:50	2.61		25	31.0		2.86	9.35	pyruvate 11:37
L	12:35	3.14	12.0	7	12.0		1.32	5.96	7.5 gm. sodium
7/10/40	12:50	2.79	12.0	12	28.0		2.94	8.81	pyruvate 12:38
T	11:27	4.79	28.0	25	9.0		1.37	3.26	10 gm. sodium
7/11/40	11:40	4.08	19.0	16	18.0		4.13	8.10	pyruvate 11:30

In table 2 are (column 2) the ratios between the blood flow during hypoglycemia and before the injection of insulin; (column 3), the observed arterio-venous oxygen difference; (column 4), the arterio-venous oxygen difference corrected for blood flow (column 2 × column 3) and column 5 contains per cent brain metabolism (column 4 ÷ control). The results of the injection of sodium lactate and sodium pyruvate are also tabulated (table 3). In these experiments neither the arterio-venous oxygen difference nor the cerebral blood flow changed markedly.

[3] $\frac{\text{Control} - \text{observed value}}{\text{observed value}}$ = per cent change, e.g., table 1, Br, 6/21/40, $\frac{6.14 - 4.07}{4.07} = 51$ per cent.

Discussion. The interrelationships of the arterio-venous oxygen differences and cerebral blood flow can be brought out in a discussion of the results of a typical example. Patient Br, 6/21/40 (table 1) started with an initial arterio-venous oxygen difference of 6.14 volumes per cent and a blood sugar of 83 mgm. per cent. He then received insulin and his blood sugar fell during a period of four hours to low levels. Meanwhile his arterio-venous oxygen difference decreased gradually to 4.07, 2.16, 2.33, and 1.34 volumes per cent. If these differences were caused by more rapid blood flow, the velocity would have to increase, as seen in the 6th column of table 1, 51, 184, 168, and 358 per cent respectively. However, the observations of blood flow reveal quite the reverse. The blood flow was not faster but slower, −10, −30, −10, and 0 per cent. In none of the present experiments could the smaller arterio-venous oxygen difference during hypoglycemia be accounted for by any change of blood flow and in most cases the changes in blood flow were opposite in direction showing a slow blood flow with a low arterio-venous oxygen difference.[4] By taking into consideration both blood flow and arterio-venous oxygen difference, it is seen that brain metabolism may decrease to approximately $\frac{1}{4}$ of the original value during hypoglycemia (table 2, column 5). The second observation of Br, 7/5/40, is probably too low. In a previous publication (8) we have recorded an average value of 1.77 volumes per cent during deep coma. With an average decrease of cerebral blood flow of 7 per cent observed in the present experiments, this indicates that brain metabolism during hypoglycemia may be reduced to 24 per cent of the original.

The effects of the injection of glucose are seen in Br, 6/27/40. The arterio-venous oxygen difference rose from 1.54 to 5.32 volumes percent, while blood sugar increased from 34 to 117 mgm. per cent following intravenous administration of glucose. Such an increase might be caused either by a greater cerebral metabolism or a slower cerebral blood flow. In this observation, blood flow would have to decrease 246 per cent to cause such a change in the arterio-venous oxygen difference. Actually it decreased only 20 per cent. Thus, the greater arterio-venous oxygen difference after the administration of glucose must be accounted for in the largest part by the augmented metabolism of the brain. This striking increase in cerebral metabolism is reflected in the changed neurological condition of the patient. Within three minutes after receiving 25 grams of glucose intravenously, the patient was aroused and in contact with his environment. Depending on various factors some time is required before

[4] We have recently been informed by Dr. C. D. Aring, University of Cincinnati College of Medicine, that he has obtained similar results on cerebral blood flow during hypoglycemia though he has used an entirely different method for the determination.

brain metabolism returns to normal. In T, 6/24/40, and C, 7/1/40, full recovery had not occurred at the time the final observations were made, but in Br, 7/5/40, the brain metabolism was the same as the original.

Because of the definite changes produced by the administration of carbohydrate, Wortis and Goldfarb (9) suggested that hypoglycemia may be employed to determine whether substances other than glucose can support brain metabolism. Lactic acid and pyruvic acid, which are intermediary products of carbohydrate metabolism, were studied in this manner. In previous work (10), 20 grams of r-sodium lactate were injected into patients during therapeutic hypoglycemic insulin coma. The average arterio-venous oxygen difference was 2.71 volumes per cent during coma. Five to twenty minutes after the injection of lactate the arterio-venous oxygen difference had increased to 4.01 volumes per cent and 25 to 50 minutes afterwards to 4.87 volumes per cent. This increase in cerebral metabolism was not adequate to arouse the patients from coma. Similar results were obtained with sodium pyruvate (11) which increased the cerebral arterio-venous oxygen difference from 2.90 to 4.06 volumes per cent. It is nevertheless possible that in these experiments there might have been a greater increase in cerebral metabolism but a larger arterio-venous oxygen difference was prevented from developing by a more rapid blood flow through the brain. For that reason experiments were performed in which in addition to determining the arterio-venous oxygen difference before and after the injection of lactate and pyruvate, observations of cerebral blood flow were also made. The injection of racemic sodium lactate (Br, 7/8/40) produced no significant change either in the arterio-venous oxygen difference, 1.63 and 1.93 volumes per cent, or in the cerebral blood flow despite the fact that lactic acid increased in the arterial blood from 13 to 40 mgm. per cent as may have also occurred in other tissues including the brain. Probably some lactic was converted to glycogen in the liver and left that organ to reënter the blood stream as glucose (12), but the rate of entry of this hepatic glucose into the blood was not rapid enough to satisfy the tissue demands augmented, as they were, by the injection of insulin. Blood sugar, therefore, did not increase. In any case lactic acid did not affect brain metabolism. Similar results were obtained with pyruvic acid (Br, 7/10/40). Though the pyruvate content of the blood increased, the arterio-venous oxygen difference and cerebral blood flow remained unchanged within the error of the methods. Arterial blood sugar was unaltered. Pyruvate rose from 1.50 mgm. per cent to 2.86 mgm. per cent and a significant amount of pyruvate was changed to lactate which increased from 14 mgm. per cent to 31 mgm. per cent. The increase of the pyruvate was responsible in part for the somewhat greater rise of bisulfite binding substances. Thus despite greater concentrations of both pyruvic

and lactic acids, brain metabolism was not stimulated sufficiently to arouse the patient. Maddock et al. (13) observed that pyruvate was not able to reëstablish brain waves during hypoglycemia in hepatectomized dogs.

These observations are in striking contrast with those obtained with glucose in which the administration of 8 grams or even 4 grams resulted in the stimulation of brain metabolism and the awakening of the patients (14). These results do not mean that lactic and pyruvic acids are not oxidized by the brain but that their oxidation occurs at a rate which is too slow to support cerebral functions.

Lactate and pyruvate are equal to glucose in ability to increase the oxygen uptake of *excised* cerebral tissues. Apparently a change has occurred in these tissues which renders lactic and pyruvic acids susceptible to more rapid oxidation. The period of anoxia after the sacrifice of the animal as well as the slicing or mincing of the tissues produces an increase of permeability which may permit lactic and pyruvic acids faster access to the respiratory enzymes. The slower utilization of lactate and pyruvate during hypoglycemia may be due to the more rapid diffusion of glucose in the brain. In the present experiments the injected lactate was not absorbed in amounts adequate for demonstration by comparison of its concentrations in arterial and venous blood. The lactic acid produced in the cell does not encounter such a barrier. When the level of lactate is elevated considerably above the normal value, the absorption of lactic acid is revealed even in a single circulation through the brain of a normal (15) or diabetic animal (16). Despite such high concentrations of lactate, patients were not aroused from hypoglycemic coma (10). The possibility remains that lactic and pyruvic acids may be oxidized more slowly than glucose *in vivo*. It would then be necessary to seek a second path for glucose metabolism not involving the intermediary stages of lactic and pyruvic acids. Such a path has been demonstrated by the use of various inhibitors: nicotine (17), hydroxymalonate (18), glyceraldehyde (19), and iodoacetate (20). These substances interfere with the oxidation or formation of lactic acid without stopping the oxidation of glucose. An explanation of the present results is at hand if the second path of oxidation is more rapid than the one including lactic and pyruvic acids. Thus two factors may be operative in facilitating a rate of utilization of glucose more rapid than that of lactic and pyruvic acids. The brain cells may be more permeable to glucose and once glucose gets into the cell its rate of oxidation may be faster.

SUMMARY AND CONCLUSIONS

The cerebral arterio-venous oxygen difference and cerebral blood flow were determined on patients with schizophrenia during insulin hypoglycemia. The changes in blood flow during insulin hypoglycemia un-

complicated by convulsions are not of great magnitude and usually exhibit a slight diminution, averaging −7 per cent. Thus, a decrease in the arterio-venous oxygen difference during insulin hypoglycemia is caused by an impaired cerebral metabolism which may be reduced to about $\frac{1}{4}$ of the original value. The injection of glucose during hypoglycemia has no marked effect on the brain blood flow but the arterio-venous oxygen difference increases. This indicates that the cerebral metabolic rate rises though full recovery may not occur immediately. Lactate and pyruvate are not as effective as glucose in restoring brain metabolism. This phenomenon is discussed briefly.

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OBSERVATIONS ON THE RÔLE OF THE THEBESIAN VEINS AND LUMINAL VESSELS IN THE RIGHT VENTRICLE[1]

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Since Vieussens (1) and Thebesius (2) first demonstrated the existence of minute vessels connecting the coronary vessels with the chambers of the heart, many investigators have studied the anatomy and function of these channels. Wearn and Zschiesche (3) showed that there are two sets of vessels, namely: 1, the Thebesian veins which join the coronary veins with the ventricular cavities, and 2, the luminal vessels which connect the coronary arteries with the ventricular chambers. The question as to whether these vessels conduct blood from the cardiac chambers into the myocardium in the normal heart has not been answered to our satisfaction. Accordingly, experiments were designed to study the direction of blood flow in those vessels which communicate with the right ventricle.

Method A. Dogs were anesthetized with morphine and sodium pentobarbital and their chests were opened under artificial respiration. The hearts were exposed and suspended in a pericardial cradle. The blood was rendered non-coagulable with chlorazol fast pink or pontamine fast pink (0.3 gram per kilo). Ventricular pressures were recorded with large needles connected to Gregg optical pressure manometers (4). The circulations of the right and left hearts were separated with a special cannula, figure 1, inserted into the pulmonary artery. It was arranged so that blood flowed from the pulmonary artery, *PA*, through stopcock *A*, and then into the lungs. About 250 cc. of a 20 per cent aqueous solution of Higgins' waterproof India Ink, or the same volume of a 2 per cent aqueous solution of Berlin Blue was injected into the right ventricle. These substances were injected by gravity at a pressure of about 60 mm. Hg. At the moment the injection was started stopcock *A* was turned one-quarter turn and the mixture of blood and dye from the pulmonary artery, *PA*,

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flowed into the rubber bag, *RB*, and displaced an equal volume of oxygenated, heparinized blood from chamber *BL* into the lungs. During the injection continuous right and left ventricular pressures were recorded. The hearts were stopped with 10 to 15 cc. of 5 per cent KCl injected into the left ventricle. After 10 to 15 progressively smaller beats the hearts came to a complete standstill. Experiments in which fibrillation occurred were not included in the series. Each heart was removed carefully, inspected, fixed in formalin, and studied microscopically.

Results. In six successful experiments, with right ventricular systolic pressure lower than left ventricular systolic pressure, there was no gross

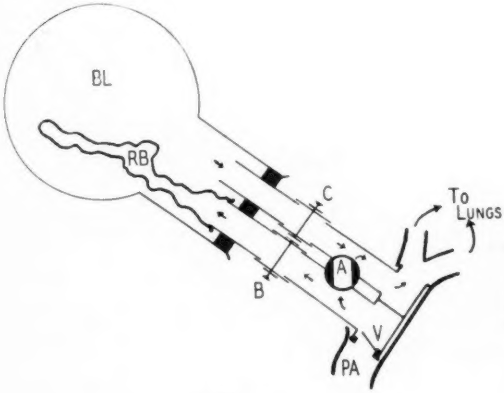

Fig. 1

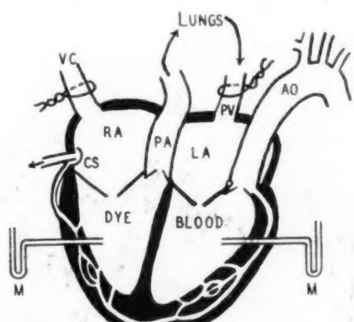

Fig. 2

Fig. 1. Diagram of special pulmonary cannula inserted into the main pulmonary artery. *A*, stopcock to allow blood to flow directly through cannula. *B* and *C*, clamps on tubes leading to and from chamber *BL* containing a large rubber bag, *RB*, and oxygenated heparinized blood. *V*, one-way rubber flap valve. *PA*, pulmonary artery.

Fig. 2. Diagram of circulation in method B. *PV*, ligature about pulmonary veins. *VC*, ligature about vena cava. *M*, *M*, manometers for measuring ventricular pressures.

injection in any of the hearts. Microscopically, occasionally two to five small vessels on the right side of the intraventricular septum were filled with dye or ink. Rarely there were a few small areas of myocardial capillary injection and more frequently a few larger injected vessels resembling sinusoids or venules. In no case was ink or dye found in large coronary arteries or veins. (See fig. 3.) In one fibrillating heart injection of dye into the right ventricle under pressure of 60 mm. Hg produced a fair amount of capillary injection in both the right and left ventricles.

Method B. Because of the great technical difficulties of the former method and in order to be able to vary the right and left ventricular pres-

sures independently, the following preparation was made: a ligature was placed through the oblique pericardial sinus about the pulmonary veins and superior and inferior vena cava. (See fig. 2.) About 250 cc. of either India Ink or Berlin Blue was injected into the right ventricle by gravity with a pressure of 60 mm. Hg. Simultaneously, from 100 to 400 cc. of oxygenated, heparinized blood was injected into the left auricle under the same pressure. When injections were started the ligature about the great veins was drawn tight, thereby separating the circulation of the right and left hearts. Continuous ventricular pressures were recorded as before. Ventricular pressures were controlled by changing the rate of the injection. Usually right ventricular pressure was maintained below

Fig. 3

Fig. 4

Fig. 3. Section through the heart of a typical experiment done by method A, showing lack of myocardial injection.

Fig. 4. Section through the heart of a typical experiment done by method B, showing complete injection of sinusoids, venules and capillaries.

left ventricular pressure, but in a few experiments right ventricular pressure was caused to exceed left ventricular pressure. In still other experiments these pressure relations were changed during the injection. The hearts were stopped, fixed, and studied as before.

Results. The results of typical experiments are shown in table 1. As in method A there was no gross or microscopic myocardial capillary injection in hearts in which right ventricular systolic pressure was below left ventricular systolic pressure. In four experiments in which the right ventricular systolic pressure was below the left ventricular systolic pressure during the first part of the injection there was no gross injection of the

hearts. In five other experiments in which the right and left ventricular systolic pressures were equal there was microscopically partial injection of venules, sinusoids and capillaries of the interventricular septum. In three of these experiments the coronary sinus was cannulated and the accessory cardiac veins were ligated to preclude any injection through the coronary veins.

When the right ventricular systolic and diastolic pressures are made to exceed the left ventricular systolic and diastolic pressures respectively

TABLE 1

EXPT. NO.	PRESSURES		REMARKS
	R. V.	*L. V.*	
	mm. Hg	*mm.* Hg	
1 (a)	28/2	95/2	Control
(b)	50/3	125/2	No gross injection
(c)	50/3	60/2	No gross injection
(d)	45/10	58/3	No gross or microscopic injection
2 (a)	26/0	100/0	Control
(b)	26/0	45/0	No gross injection
(c)	68/28	60/40	Good injection in patches of septum and right ventricle. No injection of left ventricle
3 (a)	15/0	75/3	Control
(b)	40/12	80/10	No gross injection
(c)	110/45	95/10	Heart grossly injected
(d)	85/40	48/5	Complete injection of septum, right and left ventricles grossly and microscopically
4 (a)	19/2	100/7	Control
(b)	28/3	138/3	No gross injection
(c)	50/5	75/7	No gross injection
(d)	67/10	38/8	Complete injection of septum, right and left ventricles grossly and microscopically

the picture is entirely different. Within three to four heart beats the injection material appears, first in the apical veins over the interventricular sulcus. Quickly the heart always becomes markedly discolored and remains so as long as these pressure relations are maintained. Microscopically, there is complete injection of myocardial capillaries, venules, and sinusoids in both ventricles and septum (fig. 4 and fig. 5). The left ventricular cavity usually contains a small amount of the injection material. Although the coronary veins are grossly filled with this material the coronary arteries contain only a small amount.

Discussion. Our experiments show that when India Ink or Berlin Blue is introduced into the isolated right ventricle of the beating heart, capillary injection does not occur when the right ventricular systolic pressure is below the left ventricular systolic pressure. However, these substances produce complete capillary injection if the right ventricular systolic and diastolic pressures exceed left ventricular and diastolic pressures respectively. That these substances are capable of producing com-

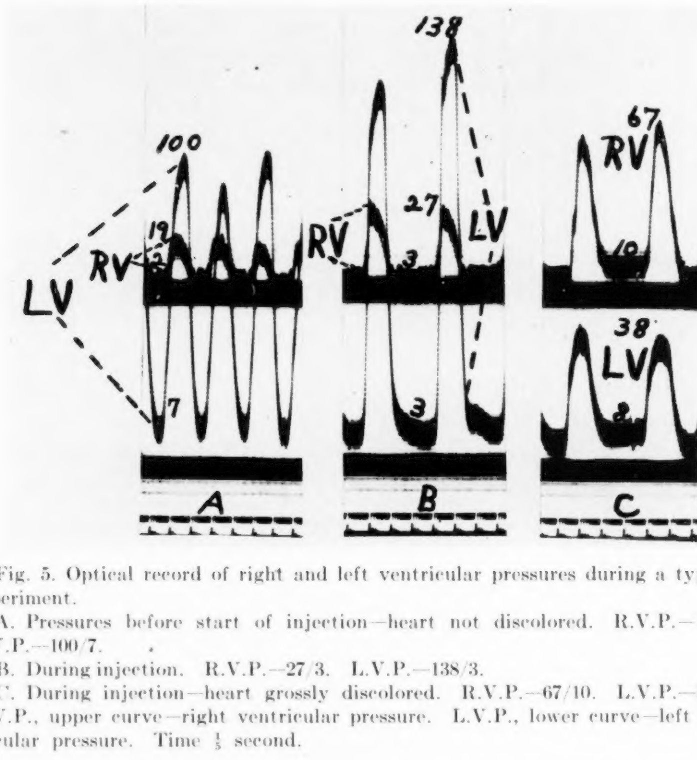

Fig. 5. Optical record of right and left ventricular pressures during a typical experiment.

A. Pressures before start of injection—heart not discolored. R.V.P.—19/2. L.V.P.—100/7.

B. During injection. R.V.P.—27/3. L.V.P.—138/3.

C. During injection—heart grossly discolored. R.V.P.—67/10. L.V.P.—38/8. R.V.P., upper curve—right ventricular pressure. L.V.P., lower curve—left ventricular pressure. Time $\frac{1}{5}$ second.

plete injection is shown by the fact that always in method B the capillaries of the lungs were completely injected. Also, in control experiments, the hearts were completely injected when these substances were injected into the right or left ventricle.

From our results we conclude that in the normally beating dog heart the myocardium receives no nourishment from the right ventricle through either the thebesian veins or luminal vessels. Bohning, Jochim and Katz (5) concluded from their experiments that myocardial nourishment may

occur through thebesian veins or luminal vessels. We believe their work does not demonstrate critically this fact for these reasons: 1, since they perfused the coronary arteries from a second dog the time and pressure relations between the ventricles and coronary arteries were not normal; 2, since they did not separate the circulations of the right and left ventricles it is impossible to say from which ventricle nourishment might occur, and 3, their published data show very little injection of the myocardial capillaries.

While it is possible that the myocardium may be nourished through thebesian veins and luminal vessels, our experiments show that if such nourishment does occur it does not have its origin in the right ventricle of the normally beating heart.

SUMMARY

The rôle of the thebesian veins and luminal vessels of the right ventricle was studied by injecting India Ink or Berlin Blue into the isolated right ventricle of the beating heart.

There was no gross or microscopic injection of the myocardium when right ventricular systolic pressure was below left ventricular systolic pressure.

Gross and microscopic myocardial injection occurred only when right ventricular systolic and diastolic pressures exceeded left ventricular systolic and diastolic pressures respectively.

Therefore, in the normally beating heart myocardial nourishment does not occur through these channels from the right ventricle.

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THE INHERENT INADEQUACIES OF THE DOUBLE HISTAMINE TEST FOR STUDIES ON PEPSIN SECRETION

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A lack of agreement exists regarding the output of pepsin in man and dog after histamine given subcutaneously at intervals of half to one hour. The data and conclusions of several groups of investigators (1–4) indicate that in *human subjects* there is an increased output of pepsin in response to both doses of histamine and that histamine stimulates pepsin secretion. A second group (5–9), who used *canine subjects*, obtained results that indicate that an increased pepsin output does not always occur after the second dose of histamine and that when there is an increased pepsin output after the first and occasionally after the second dose, it is to be regarded as due to a "washing out" of the pepsin in the lumen of the glands and not to stimulation of the pepsin producing cells. Before ascribing the discrepancy to a species difference, it was decided *a*, to investigate the validity of existing data on the response of dogs to the double histamine test, using new techniques; *b*, to learn whether artifacts in the pepsin response occur due to an unfavorable pH of the gastric juice at the time of collection, and *c*, to determine whether "washing-out" processes constitute an actual complication in this test. The new techniques to which we refer are 1, the hemoglobin method for pepsin determination, and 2, the use of dogs prepared surgically with vagotomized pouches of the entire stomach.

PROCEDURE AND METHODS. *The double histamine test.* Dogs prepared with vagotomized pouches of the entire stomach were the subjects. All food was withheld for 18 hours previous to an experiment. After draining any residual juice, the continuous secretion over two 1-hour periods was collected for control purposes. Each dog then received subcutaneously 1 mgm. of histamine dihydrochloride freshly dissolved in physiological saline. Collections were made into graduated centrifuge tubes every 15 minutes for an hour. At this time the dose of histamine was repeated and the collections continued for 1.25 hours.

All samples were analyzed individually for pepsin, free and total acidity. Pepsin was determined by the Beazell modification of the hemoglobin method (10) first described by Anson and Mirsky (11) and used by Nor-

throp (12) in his studies on crystalline pepsin. It has the advantage of being very sensitive and is more reliable than any other method employed in our laboratory. All determinations were made in duplicate, accompanied by a blank test on the inactivated juice. Since preliminary tests showed that gastric juice does not lose any peptic activity if stored in the ice box for several months, our samples were stored overnight in the ice box and the pepsin determinations made the next day. Free and total acidity were determined by titration with N/40 NaOH using Töpfer's and phenolphthalein as indicators.

Total pepsin output has been the major consideration in this work. It is the product of pepsin concentration and the volume, thus representing both variables. The results are expressed graphically as the mean curves of the arithmetical averages on the individual animals. We have preferred to use the milli-unit when expressing concentration as it enables us to express the activity per cubic centimeter as a whole number. All important trends shown by the data were investigated for statistical significance. The criterion of significance was a critical ratio of at least 3.

Method for studying effect of pH on peptic activity in gastric juice. Dog gastric juice collected under conditions of uniform secretion at pH 0.9 to 1.3 and known to have a good peptic activity was used. Aliquots were exposed for exactly 15 minutes to various pH values by diluting with an equal volume of 0.1 N HCl, water, or diluted 0.1 N NaOH. The exposure was terminated by starting the pepsin determination using 1 cc. of the diluted gastric juice in 5 cc. of the hemoglobin substrate. The pH of the digestion mixture was thereby not significantly altered from 2.2 (Coleman glass electrode). The activity manifest at the various pH values was related to that when water or 0.1N HCl was used as the diluent.

Procedure for studying possibility of "washing out." After obtaining two 1-hour samples of basal secretion, continuous histamine stimulation was inaugurated, wherein each animal received a small constant dose of histamine every 10 minutes. After 2 hours, during which a uniform secretory rate had become established, a single large dose equivalent to 7 of the 10-minute doses, was given all at once. No more histamine was given until an hour later, when the continuous stimulation was resumed and continued for another hour. The gastric juice was collected for analysis at 20-minute intervals.

DATA AND DISCUSSION. *Can it be demonstrated that in the dog there is an apparent increased output of pepsin in response to repeated hourly injections of histamine?* The results of 24 double histamine experiments, representing 6 on each of 4 animals, appear as the mean curves of arithmetical averages in figures 1 and 2. The concentration of pepsin as well as the output of pepsin *increased* in response to *both* injections of histamine. When the total hourly outputs of pepsin are studied (fig. 3) those following

both histamine injections are significantly larger than the control. Statistical analysis demonstrates a significant homogeneity between the responses of both experimental hours and again between those of the control hours.

Comment. Thus our findings with respect to the dog confirm those of Alley (7) and bring the observation in man and dog into mutual agreement. Analysis of the data of Vineberg and Babkin (6) and Toby (9) reveals that they were considering pepsin concentration only. If the pepsin output is calculated from their data, their findings are in agreement with ours. Bowie and Vineberg's work (8) with anesthetized dogs is also

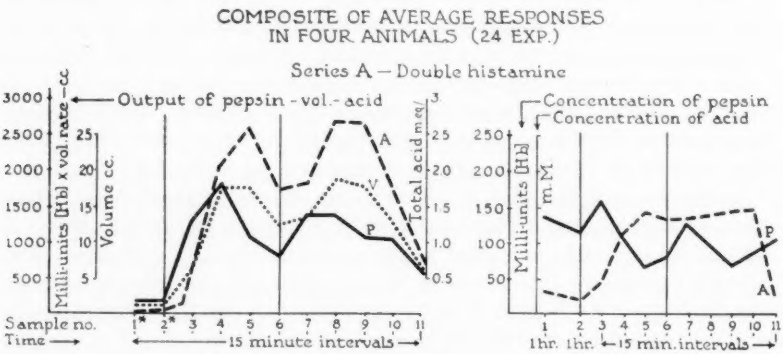

Fig. 1 Fig. 2

Fig. 1. The average output responses under the double histamine test. A = acid output in milli-equivalents; V = volume output in cubic centimeters gastric juice; P = pepsin output in milli-units pepsin (Hb). Vertical lines at 2 and 6 indicate time at which 1 mgm. histamine was injected. * denotes values calculated to a fifteen minute basis from the hourly data.

Fig. 2. Average curves representing concentration of pepsin, P, in milli-units (Hb) per cubic centimeter and concentration of acid, A, in milli-equivalents per liter, obtained in response to the double histamine test.

in agreement if the hourly outputs for the first 2 hours only are compared. In 10 experiments, Gilman and Cowgill (5) failed to observe any consistent increase in pepsin output in response to the second injection of histamine. This is not supported by Goodman (15) or ourselves.

The greater number and uniformity of these data, however, neither establish nor invalidate either interpretation (stimulation vs. mechanical washing-out) of the mechanism by which histamine supposedly influences pepsin output. The nature of this test neither proves that washing-out processes are an actuality, nor excludes such from entering into the picture during the second hour as a result of the retarded flow at the end of the first. Moreover the statistical homogeneity of the significantly increased

total hourly outputs during the experimental hours can be viewed from at least two other aspects which are exclusive of a washing-out process being concerned. These aspects are *a*, that the chief cells have been more productive and to an equally intensive degree during both experimental hours; or *b*, that the low basal outputs of the basal secretion are artifacts due to inactivation of the pepsin that is continually being secreted.

Is it possible that in low gastric acidities considerable pepsin is inactivated during the periods of collection? It is well known (13, 14) that pepsin, either in the gastric juice or in active mucosal extracts, is rapidly and irreversibly destroyed by titration to neutrality. It has not been heretofore realized that pepsin, as it occurs in the gastric juice is more severely in-

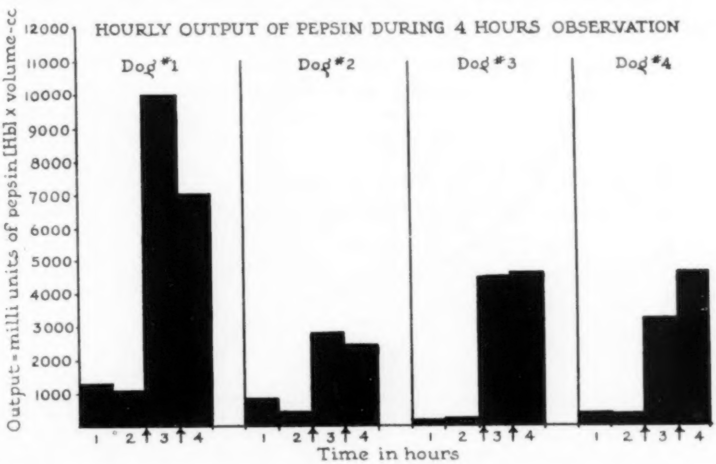

Fig. 3. Total hourly pepsin outputs of four dogs obtained under double histamine stimulation. Hours 1 and 2 are control hours. Arrows indicate the time of injection of 1 mgm. of histamine.

activated by pH changes in the range between 2.0 to 4.0 than in the range between pH 4.0 to 8.0.

The results of 38 experiments on gastric juice from 3 different dogs are presented in figure 4. In every instance a marked loss of activity was noted when the exposure was still as strongly acid as pH 2.0, amounting to approximately 30 per cent. At pH 3.5, which with Töpfer's still indicates the presence of some free acidity, the loss of peptic activity was about 60 per cent of the original. We believe that this evidence points to the necessity of collecting gastric juice intended for pepsin study under conditions where a pH of 0.9 to 1.3 is sure to prevail at all times. As soon as the pepsin is exposed to a pH higher than this range, reductions in the mani-

fest activity are invited, and the physiological efforts of the chief cells with regard to peptic output will be grossly underestimated.

That such conditions do not prevail in the double histamine test is demonstrated by the acidity curve in figure 2. If it were possible to obtain the pH on the scanty basal secretions and to know the time any given fraction of pepsin had been exposed to that pH, it is conceivable that the difference between the basal output and histamine output might be completely obliterated.

Can washing-out processes be demonstrated to be possibly involved in the double histamine test? The results of one experiment on each of 4 dogs is given in the form of average curves in figures 5 and 6, and as average hourly outputs in figure 7. It can be seen that under continuous histamine stimulation there is an induction period of one hour, after which 6 basal

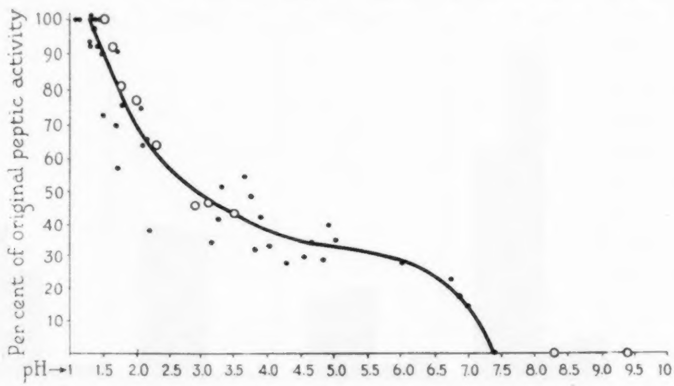

Fig. 4. Curve showing loss of peptic activity with increasing pH. Circles represent points obtained by using U.S.P. pepsin powder (Merck). The solid points are the values obtained by using dog's gastric juice.

doses, given one every 10 minutes, maintained a uniform secretory rate of uniformly high acidity with a remarkably constant total pepsin output. The response to 7 times the basal dose given as one dose at the start of the fifth hour was a decreased hourly output of *both* volume and pepsin. The volume-rate and pepsin output per minute had become reduced to half by the end of the hour. The resumption of the continuous histamine not only revived the secretory rate, but brought forth a clearly increased pepsin output. However, examination of the hourly outputs (fig. 7) supports the view that this augmented output may be the result of pepsin accumulation in the preceding hour due to the decreased volume-rate at the end of that hour. The average hourly output under continuous histamine (hrs. 3 and 4) was 4511 milli-units; that of the fifth and last hours was 4639 milli-units. These very similar averages speak for themselves.

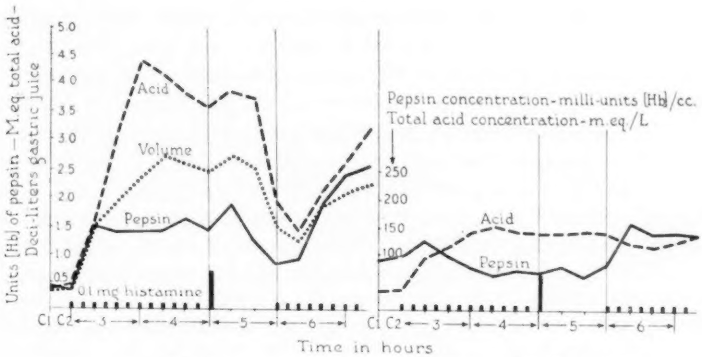

Fig. 5 Fig. 6

Fig. 5. Average total output curves of acid, volume and pepsin under continuous histamine (hrs. 3, 4 and 6) and under single histamine (hr. 5). Acid is expressed in milli-equivalents; volume as deciliters; pepsin as units (Hb). Small bars along the bottom indicate time and size of histamine dose.

Fig. 6. Average concentration curves of acid and pepsin during continuous histamine (hrs. 3, 4 and 6) and single histamine (hr. 5). Acidity is expressed as milli-equivalents per liter; pepsin is expressed as milli-units (Hb) per cubic centimeter.

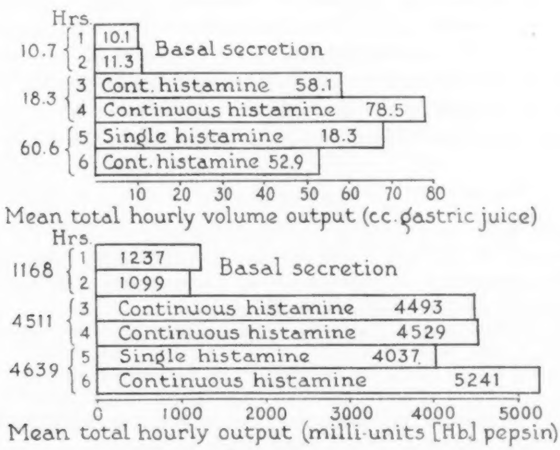

Fig. 7. Average total hourly outputs of volume in cubic centimeters gastric juice (upper) and average total hourly output of pepsin in milli-units (Hb) (lower) under continuous histamine (hrs. 3, 4 and 6) and single histamine (hr. 5).

Summary. We believe that the lack of agreement regarding the interpretation of the effect of histamine on pepsin response has its basis in

the inadequacies of the experimental approach rather than in errors or insufficiencies of the data obtained therefrom. We have found that partial inactivation of pepsin must be expected when the latter is secreted with gastric juice of low acidity, as occurs in several phases of the double histamine test. Because all deductions relative to the *amount* of pepsin liberated are made on the basis of its manifest activity at the time of collection, this factor is of great importance in the quantitative evaluation of chief cell activity. Also, complications exist which appear to be the result of accumulation and subsequent washing-out and which are unavoidable when single hourly doses of histamine are given. The double histamine test therefore has no utility in the quantitative study of pepsin secretion; this is especially true when the question of whether histamine stimulates pepsin-cell activity is involved.

CONCLUSIONS

1. In dogs with a vagotomized pouch of the entire stomach the hourly injection of histamine, or the application of the double histamine test, increases the output of pepsin apparently.

2. For the purpose of determining whether histamine stimulates pepsin production or only mechanically promotes the elimination of preformed pepsin such evidence is completely indifferent.

3. Two limitations of the double histamine test for the quantitative study of pepsin secretion have been detected. They are: *a*, artifacts due to pH inactivation occur in gastric juice collected at low and irregular acidity which are still acid to Töpfer's reagent; and *b*, artifacts due to the possibility of a washing-out process are unavoidable when great irregularities in the gastric secretory rate occur as they do in the double histamine test.

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THE STORAGE OF CARBOHYDRATE FOOD

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The largest proportion of the calories comprising the diet of man, most rodents and all herbivorous animals is in the form of carbohydrate. The ability of the mammalian organism to store carbohydrate as such is very limited. No one would deny that carbohydrate food in excess of the requirement over relatively long periods is stored as fat but the idea is prevalent throughout the metabolic literature that oxidation and deposition as glycogen accounts for almost all of the carbohydrate food ingested under ordinary circumstances. This is true for the conditions under which Cori and Cori (1) carried out their balance studies. They made their observations over the short period of 4 hours and administered sugar under conditions (2) which give absorption rates less than half those which are possible under more natural conditions (3). There are many circumstances under which even a rough calculation will show that there is an excess of ingested carbohydrate which cannot be promptly catabolized or retained as such and which therefor must be stored as fat. This formation of fat from carbohydrate is a normal process in all well fed animals when their glycogen stores become filled. Burr and his co-workers (4, 5) have presented evidence obtained with the respiratory quotient for carbohydrate storage as fat in normal rats and in a more marked degree in rats suffering from a deficiency of the essential fatty acids. Except for this we have little information as to factors which may influence the amount of ingested carbohydrate stored as fat and no direct evidence for such storage. The present study was undertaken to obtain data by direct measurements on the extent of the storage of carbohydrate food as fat over a relatively short period of time.

METHODS. The method devised to approach this problem consisted in taking pairs of litter-mate female rats and feeding and fasting them on alternate days until their food intake became approximately constant on the alternate feeding days and the respective fed and fasted weights were constant. A typical example is shown in figure 1. The food cups were left in the cages for 20 of the 24 hours during the feeding periods so that the fed weights were 4 hours postabsorptive. After equilibrium in weight changes had been established one rat of each pair was sacrificed at the end

of a feeding period (4 hrs. post-absorptive) and the other at the end of a fasting period. An experiment of this nature presents difficulties not found in the study of glycogen deposition due to the variability in the amount of preformed fat in the organism. The litter mate pairs selected in the first place were always of the same weight. At the termination of the experiment only those pairs were retained the fasting weights of which were essentially the same. This led to far more data being discarded than was retained. Ten pairs of the rats used in the data presented here were 129 days old when sacrificed and 6 pairs were 137 days of age. All of them were on the regime depicted in figure 1 for the 20 day period. Six pairs were fasted 24 hours, 6 pairs for 48 hours and 4 pairs for 60 hours before pairing on the basis of body weight and the first feeding of the special diet. The

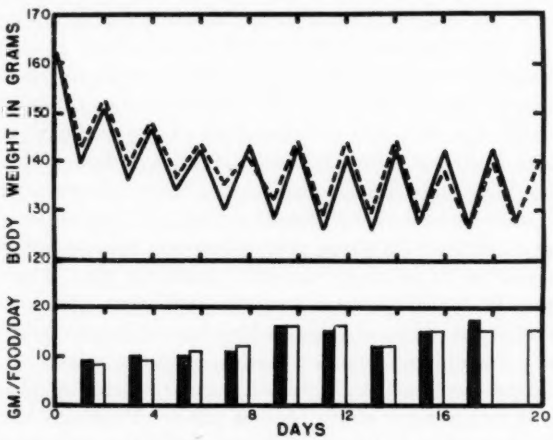

Fig. 1. Food intake on feeding days and daily variation in body weight of a typical pair of rats on regime of alternate fasting and feeding.

48 hour fast gave the best results. After fasts of 100 or more hours which might more nearly reduce the body fat to a more regular basal level the special diet is eaten poorly and the rats continue to lose weight and die during the ensuing regime.

The special diet which we used may be considered to be fat free for our purposes. It consisted of sucrose 70, casein (vitamin free, Casein Company of America) 15, brewer's yeast (Anheuser-Busch) 10 and Osborne and Mendel's (6) salt mixture 5. Vitamins A and D were supplied by feeding a small drop of a natural fish liver oil concentrate every third day. In our rough calculations of the carbohydrate intake the sucrose was considered as such. Fifty-two per cent of the yeast was taken as protein and it was assumed that 60 per cent of this and of the casein was

converted to carbohydrate although the correct figure may be higher (7). No attention was paid to the extremely small lipid content of the casein or yeast or to the possibility of fat formation from the fed protein.

TABLE 1

Relative storage as glycogen and fat of carbohydrate food

(Averages of 16 pairs of rats on alternate fasting and feeding regime)

	FASTED*			FED†		
	Min.	Av.	Max.	Min.	Av.	Max.
Initial body weight—grams	136	*169*	195	135	*169*	194
Final body weight:						
Fed—grams	125	*150*	178	130	*155*	177
Fasted—grams	112	*134*	157	116	*137*	156
Food intake—grams/rat/day‡	13	*17*	21	13	*17*	21
Body glycogen:						
Per cent	0.15	*0.19*	0.24	0.32	*0.63*	1.27
Grams/rat	0.18	*0.26*	0.33	0.44	*0.98*	1.84
Body fat:						
Per cent	1.60	*3.54*	7.05	2.50	*5.10*	8.88
Grams/rat	2.06	*4.75*	9.04	4.38	*7.92*	13.58

* One of each pair sacrificed at end of fasting day 28 hours after food had been removed from their cages.

† Second rat of each pair sacrificed at the end of a feeding day 4 hours after they had last had access to food.

‡ This is the food intake on the feeding days which of course serves a two day period.

Calculations from above data

Body weight gain during feeding days	18 grams
Food intake as grams per rat per day	8.5 grams
Food intake as calories per rat per day	33 Calories
Carbohydrate intake as grams per rat per day	7 grams
Carbohydrate intake as calories per rat per day	29 Calories
Carbohydrate deposited as glycogen per rat	0.72 ± .08* gram
Carbohydrate deposited as glycogen per rat	3 Calories
Carbohydrate deposited as fat per rat	3.17 ±0.55* grams
Carbohydrate deposited as fat per rat	29 Calories
Carbohydrate calories deposited as glycogen	10 per cent
Carbohydrate calories deposited as fat	100 per cent

* Standard error.

When the rats were to be sacrificed they were anesthetized with an intraperitoneal injection of a solution of sodium pentabarbital, the abdomen and thorax split open and the animal quickly liquidized in a hot 30 per cent KOH solution using 3 cc. of this per gram of rat. The bones are

easily filtered off with the aid of glass wool. The glycogen content of the body was found from the glycogen concentration of this solution determined in the usual manner (8). Body "fat" was determined as the petroleum ether extract of an acidified aliquot of the KOH solution and consisted of unsaponifiable lipids and fatty acids. All of the increase in the animals showing fat storage was in fatty acids.

Results. Our average figures are presented in table 1. For every pair of rats the amount of glycogen and fat in the animal was greater in the fed rat.

The food intake per rat per day in the table represents the average intake on the last four feeding days which of course cover an eight day period. The food intake per rat per day used in the calculations below this table is one-half of this figure for the last day for the *fed* group. It is just a coincidence that the averages for the last four feeding days of both groups and the last day of the *fed* group are the same. We assume that the food intake of the "feeding" day which serves for this day and the "fasting" day which follows is utilized equally during each of these days. Actually the energy expenditure is probably larger during the "feeding" day (5) but this difference would not significantly alter our calculations.

Both glycogen and fat are obviously stored after the largely carbohydrate diet (table 1). Of the two, storage as fat is by far the more important. Below table 1 calculations have been made from our average data. One should not be misled as to the accuracy of experiments of this type by the fact that our averages happen to show that we can account for all of the carbohydrate calories which are stored. Of much more importance is the ratio of carbohydrate stored as glycogen to that as fat which is 1:10.

Discussion. Under the conditions of our experiments carbohydrate food is stored chiefly as fat and only in a minor degree as carbohydrate (glycogen). We do not pretend that this must always be the case in the rat for this animal normally eats during the night and fasts during the day. In order to have suitable quantities of food, body fat and glycogen to deal with we found it necessary to double these periods. The metabolism associated with preparation for a longer fasting period may be somewhat different than with the usual 12 hour schedule but it would be surprising if much more of the stored carbohydrate was in the form of glycogen. If glycogen storage was the same there would still be 4 or 5 times as much of the carbohydrate food stored as fat as in the form of carbohydrate (glycogen). It is possible that this type of experiment might be refined so that observations could be made over the normal feeding schedule of the rat. In any case our results show the importance of transient or labile carbohydrate storage as fat and give a basis for a study of the influence of various factors upon carbohydrate storage.

SUMMARY

When rats are fed and fasted on alternate days so that their average weight and food intake becomes constant sufficient food is stored during the feeding day for maintenance during the fasting day. If the diet is composed almost entirely of carbohydrate this is largely stored in the form of fat.

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PROTHROMBIN AND FIBRINOGEN IN LYMPH[1]

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It has been a common observation that lymph collected from different sources clots on standing—an observation which indicates that all the factors necessary for clotting, including prothrombin, are present in lymph. However, we are aware of only one study of prothrombin in lymph—that of Howell (1)—in which it was demonstrated that this clotting factor is present in thoracic duct lymph of dogs. No data regarding the quantity of prothrombin in lymph are available.

In this investigation we have determined quantitatively the prothrombin content of dog lymph collected from several sites, namely, thoracic duct, portal lymphatics draining the liver, and femoral lymphatics. Our results show that prothrombin is present in lymph in considerable amounts, but in a lower concentration than in plasma. The distribution of this clotting factor between lymph and plasma is in general the same as the distribution of the total proteins between lymph and plasma.

In these experiments lymph was collected from fasting dogs except in three instances (dogs 1, 12 and 21); ordinarily the last feeding was 24 to 48 hours prior to the time of collection. Ether anesthesia was used in all experiments. Each animal was given 1 mgm. morphine sulfate per kilogram body weight subcutaneously $1\frac{1}{2}$ to 2 hours prior to the beginning of anesthesia. A short segment of the lymphatic from which lymph was to be collected was isolated and divested carefully of adjacent capillaries and other tissue. The vessel was then washed thoroughly with 0.9 per cent NaCl solution. The lymphatic was incised, and the lymph collected in a small paraffin-coated spoon which contained a measured amount of potassium oxalate solution (1.85 per cent). In the case of femoral lymph, the flow was stimulated by passive motion of the leg for a period of approximately 10 minutes. The first few drops of lymph collected after beginning the motion were discarded.

Blood samples were obtained from each dog by venipuncture (jugular). The blood (7 cc.) was mixed immediately with potassium oxalate (1 cc.)

[1] Aided by a grant from the John and Mary R. Markle Foundation. Funds for technical assistance were supplied by the Graduate College, State University of Iowa.

and the plasma, obtained by centrifugation, was used for comparison with the oxalated lymph samples from the same dog.

Prothrombin analyses were made immediately on all samples by the two-stage method of Warner, Brinkhous and Smith (2, 3). The prothrombin levels of lymph and plasma were determined simultaneously. The result of each lymph prothrombin determination is expressed in per cent, using the same dog's plasma prothrombin content as 100 per cent. In addition to these quantitative prothrombin determinations, the prothrombin level of thoracic duct lymph was measured by the one-stage method of Quick (4) in 4 of the dogs (dogs 9, 13, 16 and 17, table 1). Fibrinogen determinations were made on a few of the plasma and thoracic duct lymph samples. The method of Jones and Smith (5) was used.

The *results* obtained are shown in table 1. For thoracic duct lymph, the mean prothrombin level and the mean fibrinogen level were both approximately one-half the plasma levels of these clotting factors. The average prothrombin value of liver lymph (93 per cent) was considerably higher than in the case of thoracic duct lymph, while the average value for femoral lymph (7.6 per cent) was very much less.

It will be noted from table 1 that there are wide variations in the prothrombin content of thoracic duct lymph in different animals. The lowest value is 32 per cent, the highest 94 per cent. Similar differences will be noted in the prothrombin values based on Quick's clotting time. These variations are in sharp contrast to the relative constancy of the prothrombin level of normal dog plasma (2). A possible explanation of these differences in prothrombin level of thoracic duct lymph is suggested by the fact that it is made up of a combination of various lymphs (i.e., portal, femoral, etc.), the prothrombin content as well as the volume of which are unequal, and probably variable from time to time.

It is well established that the total protein content varies considerably in lymph from different portions of the body. In general, the protein in liver lymph is highest, that in leg lymph lowest, with average values of about 85 per cent and 28 per cent respectively of the plasma protein content. The protein in thoracic duct lymph is intermediate in amount, with average values of about 55 per cent (6, 7, 8). If one compares these values with the relative values of prothrombin and fibrinogen in lymph given in table 1, a remarkably close correspondence between them will be noted. This suggests that the process by which prothrombin and fibrinogen reach the lymph is the same as for the other lymph proteins. In the case of liver lymph, the high protein values have been attributed to the ready permeability of the liver sinusoids, allowing the rapid diffusion of the plasma into the tissue fluid and lymph in this organ. On the other hand, there is much evidence to indicate that both prothrombin (9) and fibrinogen, and probably other plasma proteins (10), are manufactured in

the liver, and the high prothrombin and high protein contents of liver lymph may be related to the hepatic formation of these substances. However, studies (8) with one of the colloidal dyestuffs, brilliant vital red, injected intravenously, have shown that the distribution of the dye between plasma and lymph is in general the same as the distribution of the total proteins. These data would suggest that the liver sinusoids are

TABLE 1

Prothrombin and fibrinogen in lymph

ANIMAL NO.	THORACIC DUCT LYMPH				PORTAL LYMPH	FEMORAL LYMPH
	Fibrinogen		Prothrombin (two-stage method)	Prothrombin (one-stage method)	PROTHROMBIN (TWO-STAGE METHOD)	PROTHROMBIN (TWO-STAGE METHOD)
	mgm. per 100 cc.	*per cent**	*per cent†*	*per cent†*	*per cent†*	*per cent†*
1			33.0			
2			32.0			
3					90.0	9.3
4					125.0	
5			50.0			
6					100.0	
7	164	43.0	59.0			
8			34.5			
9	236	50.0	41.0	23.0		5.8
10						7.5
11					82.0	
12	145	40.0	61.0			
13			43.0	28.0		
14						7.8
15					67.0	
16	259	60.0	57.0	55.0		
17	362	71.0	94.0	66.0		
18					95.0	
19	130	53.0	52.0			
20	169	51.0	65.0			
21	224	42.0	37.0			
Average	211	51.2	50.7	43.0	93.2	7.6

* Plasma fibrinogen = 100 per cent.

† Plasma prothrombin = 100 per cent.

about equally permeable to the dye and proteins, and that the permeability factor is probably more important than local protein formation in determining the high protein content of liver lymph.

SUMMARY

Prothrombin and fibrinogen are present in thoracic duct lymph of dogs in a concentration equal to approximately one-half the plasma concentra-

tions of these substances. The prothrombin content of liver lymph is nearly equal to that of blood plasma, while femoral lymph contains less than one-tenth as much prothrombin as plasma.

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A COMPARISON OF THE EFFECTS OF 11-DESOXYCORTICOSTERONE ACETATE AND 17-HYDROXY-11-DEHYDRO-CORTICOSTERONE IN PARTIALLY DEPANCREATIZED RATS[1]

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Among the principal physiologic effects of 11-desoxycorticosterone are its efficacy in maintaining the life of adrenalectomized animals (1), (2), and its influence in regulating electrolyte balance in normal as well as adrenalectomized animals (2), (3). The effect of this compound on carbohydrate metabolism is reported to be slight (4), (5), (6). In contrast, 17-hydroxy-11-dehydro-corticosterone which appears to be less effective in maintaining the life of adrenalectomized animals (7), is very potent in its effect on the carbohydrate metabolism of both normal and adrenalectomized animals (4), (5), (6), as well as patients with Addison's disease (8). The deduction has been made that these two substances differ in their principal physiologic properties. In the present investigation an attempt has been made to extend this comparison by studying the physiological changes which occurred in partially depancreatized and in adrenalectomized, partially depancreatized rats following treatment with one or the other of these crystalline adrenal cortical hormones.

METHODS. Male albino rats of the Sprague-Dawley strain were used in these experiments. The animals were partially depancreatized at a body weight of 45 to 80 grams, all of the pancreas being removed except that portion which lay between the bile duct and the duodenum. The animals were maintained on a diet of Purina Dog Chow until they had reached a weight of approximately 300 grams. They were then placed in metabolism cages and maintained on a fluid diet composed of butter 500 grams, dried egg albumin 170 grams, sucrose 325 grams, Celluflour 83 grams; Osborne and Mendel salt mixture 33 grams, sodium chloride 17 grams, Vi-Penta (Roche) 10 cc., with water added to make a total volume of 1680 cc. The diet was administered by stomach tube each morning and evening, the total daily amount being 18.4 cc. per rat. The steroid compounds were administered in sesame oil by subcutaneous injection.

[1] This study was aided by a grant to one of us (G. W. T.) from the Committee on Research in Endocrinology, National Research Council.

Twenty-four-hour specimens of urine were collected and preserved with toluene. The methods used for the analysis of urine have been described (2).

In the first series of experiments 8 partially depancreatized rats were used. Following a control period of 7 days, 11-desoxycorticosterone acetate was administered for 4 days to 5 of the animals and after a second control period of 7 days a similar quantity of 17-hydroxy-11-dehydrocorticosterone was administered for 4 days to the same animals. Two of the remaining 3 animals received only 17-hydroxy-11-dehydrocorticosterone and one animal received only 11-desoxycorticosterone acetate.

In the second series of experiments 10 adrenalectomized, partially depancreatized rats were used. Following operation the animals were permitted food ad libitum for a period of at least 4 days before force-feeding was resumed. For one week following operation, maintenance doses of aqueous adrenal cortical extract were injected and 1 per cent solution of sodium chloride was given as drinking water. Each animal was then maintained for a period of 6 or 7 days without treatment prior to the beginning of the test period. Nine of the 10 partially depancreatized animals were observed to have glycosuria prior to adrenalectomy. In all of the diabetic animals glycosuria was abolished by adrenalectomy. Six animals were treated with 11-desoxycorticosterone acetate for 4 days and, after a control period of 7 days, they were then treated with a similar quantity of 17-hydroxy-11-dehydrocorticosterone for 4 days. Two animals received only 17-hydroxy-11-dehydrocorticosterone and two animals received only 11-desoxycorticosterone acetate.

RESULTS. *Body-weight.* A striking difference was observed in the effect of the two test substances (fig. 1). All of the partially depancreatized and all of the adrenalectomized, partially depancreatized rats continued to gain weight during treatment with 11-desoxycorticosterone acetate whereas these same animals lost weight during the period in which they were treated with 17-hydroxy-11-dehydrocorticosterone. Most of the rats succumbed during, or as a result of, treatment with 17-hydroxy-11-dehydrocorticosterone and at the time of death these animals appeared to be markedly dehydrated.

Excretion of glucose and non-protein nitrogen. The daily administration of 1, 2 or 5 mgm. of 11-desoxycorticosterone acetate failed completely to induce glycosuria in either the partially depancreatized or the adrenalectomized, partially depancreatized rats. Furthermore, no increase in the renal excretion of non-protein nitrogen was noted during the period of treatment with this compound. It is of some interest to note that in the majority of animals the excretion of non-protein nitrogen was somewhat reduced (approximately 6 per cent) during and immediately (4 days) following 11-desoxycorticosterone acetate therapy. Three animals (one

partially depancreatized, and two adrenalectomized, partially depancreatized) were treated with larger doses of 11-desoxycorticosterone acetate (10 mgm. per day). In two of these animals so treated (one partially depancreatized and one adrenalectomized, partially depancreatized) a slight increase in glucose and nitrogen excretion was noted (table 1).

The daily administration of 1, 2 or 5 mgm. of 17-hydroxy-11-dehydrocorticosterone was followed by a marked glycosuria and a definite increase

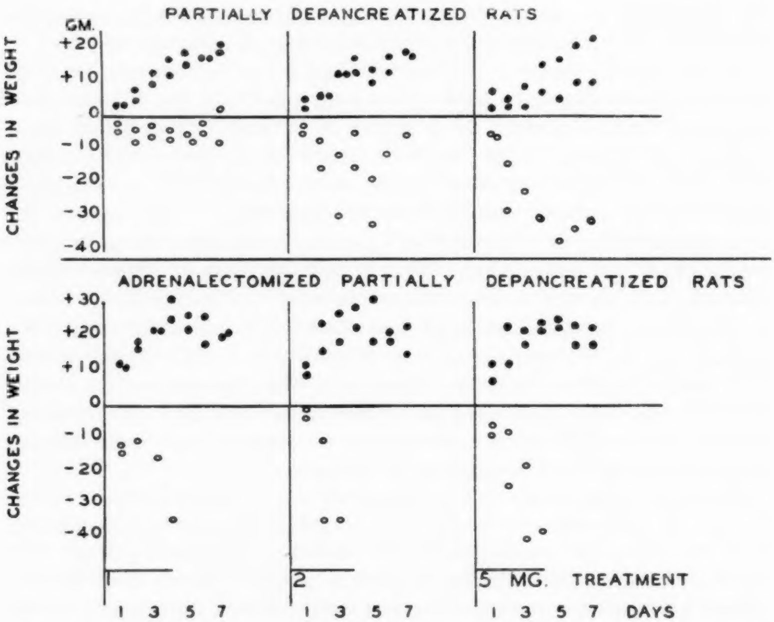

Fig. 1. A comparison between the effect of 11-desoxycorticosterone acetate and 17-hydroxy-11-dehydrocorticosterone treatment on body-weight. ●—treated with 11-desoxycorticosterone acetate; ○—treated with 17-hydroxy-11-dehydrocorticosterone.

in the excretion of non-protein nitrogen in both the partially depancreatized and the adrenalectomized, partially depancreatized animals (fig. 2). However, all of the increased glucose excretion could not be accounted for by the increase in formation of glucose from protein on the basis of a glucose:nitrogen ratio of 3.6 (6).

Excretion of ketone bodies. Ketonuria accompanied glycosuria and increased non-protein nitrogen excretion in three animals treated with 17-hydroxy-11-dehydrocorticosterone (table 2). One of these animals was

TABLE 1

The effect of treatment with large doses of 11-desoxycorticosterone acetate (10 mgm. daily) on glucose and non-protein nitrogen excretion averages

ANIMAL	PERIOD (4 DAYS EACH)	BODY WEIGHT	GLUCOSE	NON-PROTEIN NITROGEN
		grams	*grams/day*	*gram/day*
Partially depancreatized (rat 8)	Control	353	0.9	0.186
	Treated	340	3.1	0.254
	Control	366	0.0	0.187
Adrenalectomized, partially depancreatized (rat 24)	Control	326	0.0	0.145
	Treated	342	0.0	0.170
	Control	352	0.0	0.145
Adrenalectomized, partially depancreatized (rat 25)	Control	312	0.0	0.196
	Treated	337	2.2	0.202
	Control	340	0.0	0.189

TABLE 2

The effect of treatment with 17-hydroxy-11-dehydrocorticosterone on the excretion of ketone bodies

ANIMAL	HORMONE	KETONE EXCRETION, MGM. PER DAY				
		1	2	3	4	5
A. Partially depancreatized						
	mgm. per day					
Rat 3	1	0	0	0	0	0
Rat 17	1	0	0	0	0	0
Rat 3	2	0	0	0	0	0
Rat 15	2	0	10	41	0	138*
Rat 3	5	0	0	0	0	0
Rat 20	5	0	0*			
B. Adrenalectomized, partially depancreatized						
Rat 21	1	0	0*			
Rat 17	1	0	0	0	0*	
Rat 23	2	0	0	0*		
Rat 14	2	0	0*			
Rat 22	5	0	54	262*		
Rat 19	5	0	0	0	176	110*

* Animal succumbed.

partially depancreatized and two were adrenalectomized, partially depancreatized. Prior to death the carbon dioxide combining power of the blood of two of the animals was observed to be 17 and 24 volumes per

ERRATUM

Volume **132**, page 674. In figure 2 of the paper: A Comparison of the Effects of 11-Desoxycorticosterone Acetate and 17-Hydroxy-11-Dehydro-Corticosterone in Partially Depancreatized Rats, by Dwight J. Ingle and George W. Thorn, the values for "urinary glucose" are expressed in tenths of grams. They should be expressed in grams.

cent, respectively. These data suggest that diabetic acidosis was the cause of death in these animals.

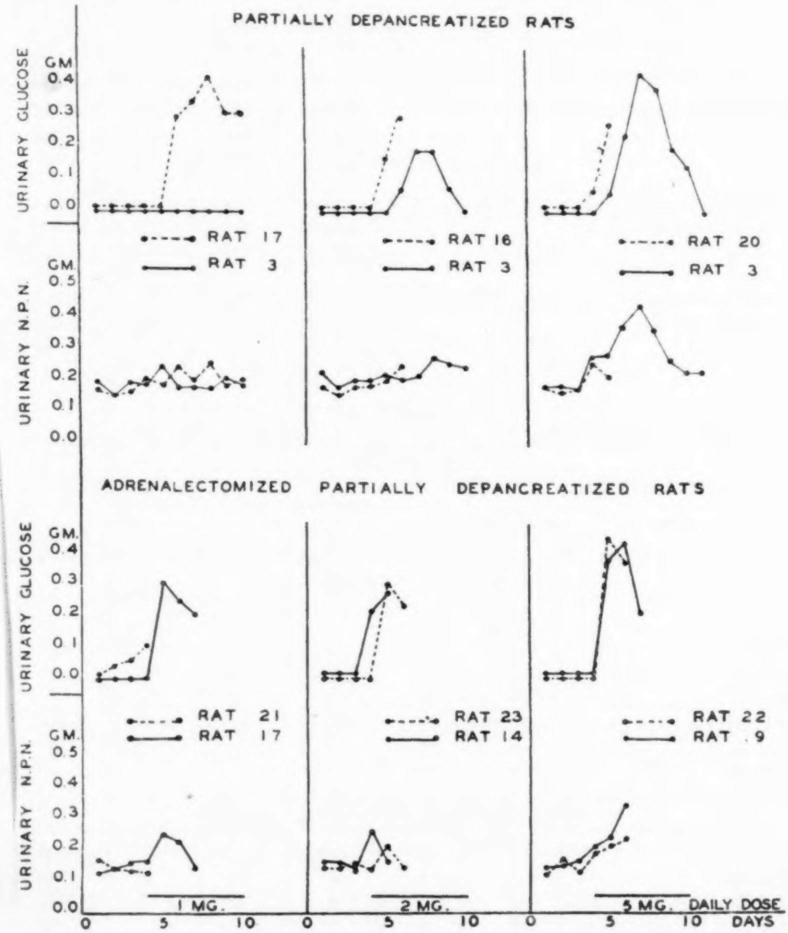

Fig. 2. The effect of 17-hydroxy-11-dehydrocorticosterone[2] on the excretion of glucose and non-protein nitrogen.

Renal excretion of sodium, chloride, potassium and inorganic phosphorus. Treatment with daily injections of 1, 2 or 5 mgm. of 11-desoxycorticosterone acetate was associated with a decrease in the renal excre-

[2] The treatment of these same animals with identical amounts of 11-desoxycorticosterone acetate failed to increase the excretion of glucose or non-protein nitrogen in every instance.

tion of sodium and chloride in all but one of the experimental animals (table 3). Equivalent quantities of 17-hydroxy-11-dehydrocorticoster-

TABLE 3

The effect of treatment with 11-desoxycorticosterone acetate or 17-hydroxy-11-dehydrocorticosterone on the renal excretion of sodium and chloride

ANIMAL NUMBER	HORMONE	RENAL EXCRETION			
		Na		Cl	
		Retention	Increased excretion	Retention	Increased excretion
		Partially depancreatized			
		A. 11-desoxycorticosterone acetate			
	mgm. per day	*per cent*	*per cent*	*per cent*	*per cent*
3	1	12		7	
17	1	7			5
3	2	11		9	
16	2	10		1	
3	5	7		0	
20	5	1		7	
		B. 17-hydroxy-11-dehydrocorticosterone			
3	1	2		4	
17	1		1	4	
3	2	4		9	
16	2		19		14
3	5		14		10
20	5	20			12
		Adrenalectomized, partially depancreatized			
		A. 11-desoxycorticosterone acetate			
17	1		17	8	
21	1	34		13	
14	2	18		12	
23	2	20		8	
19	5	41		22	
22	5	21		4	
		B. 17-hydroxy-11-dehydrocorticosterone			
17	1		6		7
21	1	30		53	
14	2	24		17	
23	2	20		20	
19	5		53		6
22	5		13	4	

one were not as effective in this respect. The excretion of potassium and inorganic phosphorus was studied in four partially depancreatized and

two adrenalectomized, partially depancreatized rats. In four of the six animals, there was an appreciable increase in the excretion of potassium during treatment with 11-desoxycorticosterone acetate. This compound had no consistent effect on the excretion of inorganic phosphorus. Treatment with 17-hydroxy-11-dehydrocorticosterone was followed by a much more striking increase in the excretion of inorganic phosphorus (table 4). The marked increase in phosphorus and potassium excretion following treatment with 17-hydroxy-11-dehydrocorticosterone appeared to reflect the increased catabolism of protein which was observed during the period of treatment with this compound. Thus it appeared that treatment with 11-desoxycorticosterone acetate had a more striking effect on the regulation of sodium and chloride balance, whereas the effect of 17-hydroxy-11-dehydrocorticosterone was more marked on phosphorus metabolism.

TABLE 4

A comparison between the effect of 11-desoxycorticosterone acetate and 17-hydroxy-11-dehydrocorticosterone treatment on the renal excretion of inorganic phosphorus and potassium in a typical case

(Rat 15—partially depancreatized)

PERIOD (4 DAYS)	RENAL EXCRETION	
	Inorganic phosphorus	Potassium
	mgm. per day	*m.eq. per day*
Control	77	1.6
2 mgm. daily of 11-desoxycorticosterone acetate	95	2.0
Control	87	1.5
2 mgm. daily of 17-hydroxy-11-dehydrocorticosterone	148	1.8

Discussion. In preparing the experimental animals for this study, it was observed that adrenalectomy was followed by a complete disappearance of glycosuria in nine of ten partially depancreatized animals. These observations confirm the studies of Long and others (4).

Treatment with 11-desoxycorticosterone acetate was much more effective than 17-hydroxy-11-dehydrocorticosterone in inducing sodium and chloride retention. Injections of 11-desoxycorticosterone acetate were also followed by a small, but definite increase in potassium excretion without a significant increase in the excretion of inorganic phosphorus. It appears probable that this alteration in potassium excretion was a reflection of the direct effect of 11-desoxycorticosterone acetate on sodium: potassium balance (3). Wells and Kendall (7) have observed that treatment with 11-desoxycorticosterone and its acetate depressed the level of potassium and elevated the level of sodium in the blood serum to a

greater extent than did treatment with 17-hydroxy-11-dehydrocorticosterone. It is probable that the glycosuria, ketosis and weight loss following treatment with 17-hydroxy-11-dehydrocorticosterone appreciably decreased the apparent sodium and chloride retaining effect of this compound. Treatment with 17-hydroxy-11-dehydrocorticosterone was much more effective than 11-desoxycorticosterone acetate in inducing glycosuria, increased nitrogen excretion and ketosis. The striking increase in the excretion of inorganic phosphorus and non-protein nitrogen which accompanied the slight increase in potassium excretion during treatment with 17-hydroxy-11-dehydrocorticosterone acetate suggests that the abnormal excretion of these electrolytes reflected the effect of this compound in increasing gluconeogenesis.

It is of considerable interest to note that not all of the increased glucose excretion which was observed following 17-hydroxy-11-dehydrocorticosterone therapy could be accounted for on the basis of increased glucose formation from protein. This observation was similar to the effect observed in phlorhizin-treated, adrenalectomized rats (6) and suggests that this substance has a direct inhibitory influence on glucose oxidation as well as on the formation of glucose from protein (6).

Summary. Partially depancreatized and adrenalectomized, partially depancreatized rats were used to compare the physiological properties of 11-desoxycorticosterone acetate and 17-hydroxy-11-dehydrocorticosterone. Treatment with 1, 2 or 5 mgm. of the latter compound was followed by glycosuria, ketonuria, an increase in the excretion of non-protein nitrogen, potassium and inorganic phosphorus, loss in body weight and ultimately resulted in the death of the animal. However, all of the increased glucose excretion could not be accounted for on the basis of increased glucose formation from protein. Treatment with equivalent quantities of 11-desoxycorticosterone acetate failed to induce glycosuria, ketonuria or increased non-protein nitrogen excretion. A slight increase in potassium excretion, unaccompanied by an increased excretion of inorganic phosphorus, was noted following 11-desoxycorticosterone acetate therapy. Larger doses of 11-desoxycorticosterone acetate (10 mgm. daily) induced glycosuria in two of three animals so treated. In contrast to the effect of 17-hydroxy-11-dehydrocorticosterone, treatment with 1, 2 or 5 mgm. of 11-desoxycorticosterone acetate was followed by a striking decrease in the renal excretion of sodium and chloride in 11 of 12 experiments.

Conclusion

Striking differences in the principal physiological effects of 11-desoxycorticosterone acetate and 17-hydroxy-11-dehydrocorticosterone were observed in partially depancreatized and in adrenalectomized, partially depancreatized rats.

Acknowledgment. The authors are greatly indebted to Dr. J. J. Pfiffner, Parke, Davis and Company, Detroit, Michigan, for supplying the 17-hydroxy-11-dehydrocorticosterone and to Dr. E. Schwenk of the Schering Corporation, Bloomfield, New Jersey, for supplying the 11-desoxycorticosterone acetate.

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CONDITIONS IN WHICH THE LIVER RETAINS LACTIC ACID

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According to Cherry and Crandall (1), retention of lactic acid by the liver does not occur in normal unanesthetized dogs that are regularly fed. Himwich (2) has confirmed these observations using anesthetized dogs, and quotes the suggestion of Crandall that fasting may be an appropriate stimulus for retention.

Because previous observations have shown that lactic acid *may* be removed from the blood by the liver (3), and because McClure (4) has suggested that an observed increase in the pH of the blood passing through the liver may be explained by lactic acid retention, it has seemed desirable to investigate the conditions under which hepatic removal of lactic acid does occur. Our studies have been carried out on normal, unanesthetized, angiostomized animals to avoid the disturbances of carbohydrate metabolism that may be produced by anesthesia and surgical procedures (1); it may be noted that previous investigations have involved indirect methods or acute experiments under anesthesia.

Methods: All experiments have been done on 12 to 15 kilo angiostomized dogs, 16 hours or more after the last meal.

"True" blood sugars were determined by Somogyi's modification of the Shaffer-Hartman method using Somogyi's second macro procedure for preparing blood filtrates. Lactic acids have been determined by a modification of the method of Miller and Muntz (5). Duplicate lactic acid analyses on twelve filtrates made from the same blood sample show a standard deviation from the mean of $\pm$ 0.8 mgm. per cent. No change of less than two milligrams per cent has been considered significant. The lactate and blood sugar values for the inflowing blood of the liver have been calculated on the basis that the hepatic artery supplies one-fourth of the total and the portal vein three-fourths.

RESULTS. Four and twenty-five one hundredths grams of *dl*-lactic acid given orally to 4 dogs produced no significant increase in blood lactic acid level or in retention by the liver (table 1-A). The control lactic acid levels in these animals are higher than in subsequent experiments, presumably

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TABLE 1

Effect of lactic acid, sodium lactate and hydrochloric acid on hepatic retention of lactic acid

+ means output by liver, − means retention

EXPERIMENT	HEPATIC RETENTION, MGM. PER CENT			ARTERIAL LEVEL, MGM. PER CENT		
	Control	30 min.	60 min.	Control	30 min.	60 min.
A. 4.25 grams dl lactic acid in 200 ml. of water, orally						
112	+2	−1	−2	28	23	22
113	+6	+5	+6	23	18	21
114	+2	+6	+4	23	23	23
115	+2	+3	0	19	14	15
116	0	−2	0	13	16	17
Average..	+2.4	+2.2	+1.6	21.2	18.8	19.6
B. 8.5 grams dl lactic acid in 200 ml. of water, orally						
127	+3	−3	0	11	28	22
128	+1	−3	0	8	16	11
129	+6	−1	−1	9	25	25
130	+4	−1	−1	9	15	17
Average..	+3.5	−2.0	−0.5	9.3	21	18.8
C. 6.5 grams of sodium lactate (dl) in 200 ml. of water, orally						
106	−2	+1	+1	9	14	11
117	+5	+3	−1	17	17	22
118	0	−3	−3	13	22	27
119	+2	+2	−1	12	25	35
Average..	+1.3	+0.8	−1.0	12.8	19.5	23.8
D. 150 ml. 2.5 per cent Na lactate (dl) intravenously at 5.0 ml./min.						
110	+5	+2	−2	7	42	26
120	0	+2	+1	11	26	18
120A	+1	+4	+2	16	27	22
E. 5.0 ml. of conc. comm. HCl in 200 ml. of water, orally						
105A	−1	0	+1	6	5	4
105B	−1	−1	−1	10	9	7
105C	+2	0	−1	14	12	9
F. 10.0 grams of $NaHCO_3$ in 200 ml. of water, orally						
102	+5	+2	+4	17	10	12
125	0	+1	0	8	53	43
126	−2	0	−1	5	6	6

because the dogs were led on chains from their cages to an adjoining room whereas in all later observations the animals were lifted from their cages and placed directly on a table.

The blood glucose showed no significant changes in these experiments. Since this is also true of most of the subsequent experiments, only noteworthy changes will be mentioned although analyses for glucose were made on every blood sample.

When the oral dosage of lactic acid was increased to 8.5 grams (table 1-B) in four dogs, the arterial level doubled, and the previous slight hepatic output of lactic acid changed to a slight retention. After 6.5 grams of sodium lactate (table 1-C) the arterial lactic acid level increased more slowly but doubled at 1 hour, and in two of the four animals there was a change toward hepatic retention that is suggestive. However, a comparable increase in blood lactate produced by the intravenous injection of 3.75 grams of sodium salt over a period of 30 minutes (table 1-D) resulted in a tendency for the liver to retain lactic acid in but one of three experiments. In general it may be said that the effect on hepatic activity of doubling the blood lactic acid is certainly not striking, and leads to no such degree of retention as is uniformly observed in fasted animals that have much lower blood lactic acid concentrations (see below). It is also evident that moderate increases in the blood lactic acid level are not necessarily associated with retention of lactic acid by the liver.

Hydrochloric acid and sodium bicarbonate were given orally in an attempt to test McClure's theory. The acid produced a suggestive decrease in blood lactic acid level but no evidence of increased hepatic retention (table 1-E). Sodium bicarbonate had no demonstrable effect on either (table 1-F).

The effect of carbohydrate deprivation was studied in 6 dogs which were fasted for periods up to 7 days or were maintained for longer periods on nothing but 100 ml. of olive oil per day. There was consistently a hepatic removal of lactic acid from the blood in these animals (table 2). In each instance the fast or fat feeding period was terminated by the administration of 20 grams of glucose, and as shown in table 2 this did not reduce the amount of lactic acid removed by the liver per unit volume of blood within the first hour after glucose. The blood lactic acid level rose after glucose administration.

As shown in table 3, the intravenous injection of physiological amounts of epinephrine for a 10 minute period roughly tripled the blood lactic acid level, and only a slight decline occurred during the next 20 minutes. In the six dogs that had had their last meal within 20 hours, epinephrine produced no demonstrable change in retention of lactic acid by the liver. This again indicates that an increase in the blood lactic acid level is not necessarily associated with hepatic retention. Dogs that had fasted 36

TABLE 2

Hepatic retention of lactic acid in starvation and following termination of fast with glucose

+ indicates output by liver, − indicates retention

EXPERIMENT	HEPATIC RETENTION, MGM. PER CENT								ARTERIAL LACTIC ACID, MGM. PER CENT							
	Hours of starvation															
	24	48	72	120	168	216	240	288	24	48	72	120	168	216	240	288
121A	−3	−4	−4	−4	−2				8	9	7	9	8			
121B	−2	−1	−4	−6	−3				11	15	12	11	12			
122*		−4		−1	−4	−3	−6	−3		11		10	8	9	15	5

20 grams of glucose after control sample taken

EXPERIMENT	HOURS STARVATION	CONTROL	30 MIN.	60 MIN.	CONTROL	30 MIN.	60 MIN.
121A	168	−2	−3	0	8	22	29
121B	168	−2	−1	−4	11	15	12
122	288*	−3	−4	−3	5	7	9
123A	408*	−7	−6	−6	12	17	23
123B	432*	−4	−6	−4	8	11	11
124	480*	−2	−2	−3	8	8	10

* One hundred milliliters of olive oil per day for varying periods of fast.

TABLE 3

Hepatic retention (or output) of lactic acid following intravenous injection of epinephrine in post-absorptive and starving dogs

Injection period 10 min.; rate 0.004 mgm. epinephrine/kilo/min. + indicates output, − retention.

EXPERIMENT	HOURS SINCE FOOD	HEPATIC LACTIC ACID RETENTION, MGM. PER CENT			ARTERIAL LACTIC ACID CONCENTRATION, MGM. PER CENT		
		Control	10 min.	30 min.	Control	10 min.	30 min.
143	16–20	+2	−1	+1	11	39	26
144	16–20	−3	+2	+2	8	28	24
145	16–20	+1	+1	+1	10	31	30
146	16–20	+1	−1	0	17	31	29
147	16–20	−1	−1	−1	8	29	33
148	16–20	0	−2	−2	8	17	16
Average		0.0	−0.33	+0.16	10.3	29.2	26.3
140	48	−2	−6	−2	9	24	22
141	36	−2	−6	−4	10	33	24
142	48	−2	−7	+1	9	40	23
149	48	−2	−3	+3	6	26	28
150	72	−1	−4	−2	6	21	25
151	72	−3	−8	−10	9	20	25
Average		−2.0	−5.7	−2.3	8.2	27.2	24.5

to 72 hours, and were with one exception showing significant hepatic retention of lactic acid, responded to epinephrine in all but one instance with definite increases in lactic acid retention by the liver when the epinephrine injection was terminated (10 min. period). Twenty minutes later (30 min. period) 4 of the dogs had returned to the pre-injection rate of removal. The fact that epinephrine increases the amount of lactic acid retained per unit volume of blood does not necessarily indicate, in the absence of blood flow determinations, an increased retention per unit time. However it should be pointed out that it is necessary to postulate an average decrease in blood flow to 35 per cent of its original value if one would explain the observed changes at the 10 minute period on the basis of blood flow variation. The hepatic output of glucose was considerably increased at the 10 minute period in most experiments, and the blood sugar level more than doubled. At the 30 minute period (20 min. after stopping the epinephrine injection) hepatic glucose output had either ceased or actual retention was occurring in 8 of the 12 experiments, and the blood sugar level had fallen sharply.

TABLE 4

Comparison of average hepatic retention of lactic acid in post-absorptive state and during fasting

	NUMBER OF DETERMINATIONS	AVERAGE RETENTION	STANDARD ERROR
		mgm. per cent	
Post-absorptive	31	−0.35	±0.51
Starvation	20	−3.6	±0.93

Discussion. Our data support previous evidence (1, 2) that lactic acid is not retained by the liver when the animal is in the post-absorptive state. The mean of 31 observations on our dogs fed not more than 24 hours previously shows a retention of 0.35 mgm. per 100 ml. of blood, which is less than its standard error of ± 0.51.

On the other hand, the liver consistently does retain lactic acid in fasted animals. The mean of 20 observations on dogs 24 hours or more after the last meal shows a retention of 3.6 mgm., with a standard error of ± 0.93. Fasting is the only procedure we have used that leads to such unequivocal results. In post-absorptive animals, doubling or tripling the blood lactate level by the oral or intravenous administration of sodium lactate, or the oral administration of lactic acid, or by intravenous injections of epinephrine, does not produce a clearly significant change in the behavior of the liver. A suggestive trend toward retention occurs when lactic acid is given orally in the larger doses.

We believe that previous evidence, supported by the data here presented, shows that hepatic lactic acid retention and conversion to glucose is not a

mechanism for maintaining acid base balance in non-fasting animals. In fact, we have been unable to obtain any definite results that can be interpreted as supporting the hypothesis of McClure (4), even when a mineral acid was given by mouth to the limit of tolerance. Our observations do not invalidate McClure's theory, but suggest that further evidence should be presented if it is to be accepted even for the condition of acidosis.

Since we do not know the effect on hepatic blood flow of the amount of epinephrine we injected, it is impossible to interpret these data in terms of hepatic lactic acid retention per unit time. It is evident that epinephrine does not significantly affect the behavior of the liver toward lactic acid in the non-fasting animal; however the increased retention per unit volume following injections of epinephrine in the fasting dog suggests that when liver glycogen reserves are low, epinephrine may bring about increased gluconeogenesis.

It has been shown previously (6) that the metabolism of fat is qualitatively different in the post-absorptive and fasting states. Lactic acid retention (and presumably gluconeogenesis from lactic acid) has now been shown not to occur in the regularly fed animal and to be present during fasting or fat feeding. It may therefore be regarded as another aspect of the special metabolism of the glucoprivic state.

SUMMARY

The non-occurrence of hepatic lactic acid retention in the non-fasting animal is confirmed. Significant lactic acid retention is present during fasting or fat feeding. The administration of lactic acid or sodium lactate by mouth, or the intravenous administration of sodium lactate or epinephrine, do not produce clearly significant lactic acid retention by the liver. Large amounts of lactic acid orally, however, result in a suggestive trend toward retention. Oral administration of acid or base does not affect lactic acid retention by the liver as estimated by our methods.

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RESPIRATORY AND METABOLIC EFFECTS OF HYPOTHERMIA

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Recent developments have revived the interest of physiologists in the effects of cold on homiothermic animals. The monograph of Benedict and Lee (1) on hibernation gives us a clear picture of the metabolic, circulatory and respiratory responses of the marmot as cold brings about the state of hibernation. Smith and Fay (2) in treating cancer have induced experimentally for the first time in man body temperatures as low as 24°C. Experiences of troops in Finland, Norway and Greece and of our own armed forces in the Arctic and in high altitudes have emphasized the need for a clear insight into such questions as adaptation to, and protection against, cold and the nature of the breakdown resulting from prolonged exposure to cold. These questions call for knowledge of metabolic, respiratory and cardiovascular responses to cold, of the relation of fatigue and of food to the ability to withstand cold, and of the protective characteristics of various types of shoes and clothing.

As an outgrowth of the treatment of cancer patients developed by Smith and Fay, an experimental hypothermic treatment of schizophrenic patients has been instituted at the McLean Hospital in Waverley, Massachusetts. The rationale of the treatment, the protocols of the cases and the therapeutic results will be reported elsewhere by Drs. Kenneth J. Tillotson and John H. Talbott (3). Briefly, the naked and lightly anesthetized patient is placed between rubberized blankets. These contain rubber coils through which a refrigerated fluid circulates. It enters at a temperature of -2 to $-5°$ during the induction period, and since heat transfer through rubber is moderately effective the skin temperature is lowered by 20°C. or more. Peripheral vasoconstriction and shivering occasionally delay the fall in rectal temperature for some hours. When it does begin to fall, regulation is effected by adjusting the flow and temperature of the circulating fluid. Within 1 to 4 hours the administered anesthetic has been dissipated; thereafter cold itself provides the only anesthesia. We have been able to make many physiological observations on these patients during treatment and are reporting here metabolic and respiratory responses.

TABLE 1

Metabolic changes in hypothermia

SUBJECT	HEIGHT	WEIGHT	AGE	HOUR OF TREATMENT	RECTAL TEMP.	RESPIRATORY VOLUME STP	RQ	CO_2 OUTPUT	O_2 USED	ESTIMATED NORMAL BASAL O_2 CONSUMPTION	OBSERVED MR/ESTIMATED BASAL MR	REMARKS
	cm.	*kgm.*	*yrs.*		*°C.*	*l./min.*		*cc./min.*	*cc./min.*	*cc./min.*		
Mrs. H. M.	165	59.0	39	3	37.2	6.3	0.92	241	262	208	1.26	Shivering
				13	25.5	9.6	0.68	223	328		1.58	
				21	29.4	16.3	0.78	343	443		2.13	Shivering
Miss S. E.	168	47.6	30	2	37.0	9.4	0.79	205	259	195	1.33	
				4	35.0	23.9	0.81	564	700		3.59	Quiet
				10	30.0	12.6	0.78	281	358		1.84	Quiet
				24	26.7	7.8	0.73	111	153		0.78	
				29	28.3	7.0	0.72	93	132		0.66	
Mr. W. D.	178	73.5	46	0	37.0	10.9	0.86	220	256	255	1.00	Before
				8	34.4	28.3	0.87	580	750		2.94	Struggling
				24	28.0	14.0	0.78	256	330		1.29	Quiet
				33	29.4	18.1	0.73	235	324		1.27	Restless
				48	31.4	16.1	0.51	195	386		1.51	Active
Miss S. J.	166	55.4	23	0	37.2	8.4	0.79	197	248	208	1.19	Before
				9	35.6	16.6	0.68	437	647		3.11	
Miss B. E.	175	67.6	25	10	34.3	14.1	0.74	392	530	232	2.28	
				24	32.8	16.3	0.75	415	556		2.40	
Mr. S. R.	177	59.4	16	1	38.3	6.0	0.69	147	214	266	0.80	Restless
				9	36.1	18.5	0.81	617	760		2.86	Struggling
				23	35.0	14.9	0.72	407	570		2.14	
Miss H. I.	163	58.1	37	8	33.0	13.4	0.79	302	383	207	1.85	
				28	32.3	4.8	0.79	87	112		0.54	
Miss P. M.	160	49.9	38	1	37.6	8.9	0.78	219	280	189	1.48	
				11	30.0	9.8	0.73	174	237		1.25	
				28	31.1	6.9	0.73	113	155		0.82	Quiet
Miss M. F.	161	54.0	25	0	38.0	6.8	1.00	160	160	199	0.80	Resting
				23	32.2	12.3	0.74	267	363		1.84	
				32	39.9	10.4	0.80	206	256		1.28	

Results. The metabolic measurements are shown in table 1. Since it was usually impossible because of the disturbed mental state to determine the basal metabolic rate before induction of hypothermia unless the

patient was anesthetized, we have incorporated in this table the records of height, weight, age and sex and then calculated from the Aub-DuBois standards the basal oxygen requirement. The observed oxygen consumption was then expressed both as cubic centimeters per minute and as a ratio, taking the estimated basal metabolic rate, BMR, as unity. Under the experimental conditions there were five instances where the observed oxygen consumption fell below the predicted basal level. In 17 instances the temperature was below 37° and yet the metabolic rate was elevated. It was commonly more than doubled in the early stages of induction, and in some instances was increased 50 per cent even when the temperature was as low as 30°. This elevation in metabolism that persists despite the falling temperature is chiefly a consequence of involuntary shivering and of voluntary muscular activity. While both are suppressed in deep

TABLE 2

Typical notes on muscle rigidity

SUBJECT	TEMPERATURE	NOTES
	°C.	
Mrs. H. M.	30.0	Restless and rigid
Miss S. E.	26.7	Body rigid
Mr. W. D.	31.1	Rigid and resistive
Miss S. J.	29.5	Short periods of restlessness and rigidity
Miss B. E.	33.1	Much quieter, relaxed
Mr. S. R.	34.8	Extremities very rigid
Miss H. I.	37.9*	Body very rigid
Miss P. M.	30.6	Limbs rigid and resistive
Miss M. F.	30.6	Abdomen board-like; body, particularly limbs, rigid

* Induction period.

anesthesia, experience indicates that hypothermia can be maintained with greater safety if cold alone is relied upon for anesthesia. While shivering may persist even at 30°, the attacks become less frequent and are usually less intense below this temperature. Voluntary movements, sometimes requiring restraint, continue at 30° and even below. Our metabolic measurements probably do not reflect the greatest periods of activity, for then it is impossible to keep the face mask in place.

In addition to the voluntary movements and the shivering there was another type of muscular contraction which probably increased the metabolism a small amount. In every patient exposed to low temperatures some flexor muscles at times assumed a state of contraction. It was often difficult to straighten the arm enough to draw blood from the antecubital vein, and the protocols contain many notes such as those quoted in table 2. This state can hardly be one of voluntary contraction, since the performance of so much static work would bring on exhaustion reflected by a degree

of hyperpnea and lactic acid accumulation surpassing those seen in any of these patients. Neither is it properly described as "cold rigor," for in isolated muscle the lower the temperature, the slower the development of rigor, as shown by Baumann (4). It does not depend simply on body temperature, since it may be present when the temperature is only slightly reduced and absent at low temperatures. Its mechanism remains to be elucidated.

The respiratory quotients (table 1) must be viewed with some reserve since the patients were not always in a steady state preceding and during the period of measurement. Although a considerable amount of glucose solution was given by stomach tube (usually 10 grams per hour), there was a tendency toward a decreased utilization of carbohydrate. This was seen in six out of seven patients on whom an observation was made early in the treatment. This trend, assuming its reality, may depend on depleted reserve of carbohydrate or, on the other hand, on inability to use it. In this connection it is reported (5) that an effective means of rendering the dog's liver glycogen-free is induction of violent shivering by extreme cold. This suggests that early depletion of carbohydrate rather than incapacity for its utilization is involved. There is a significant additional fact: acetonuria may be intense. This implies that there is no unusual capacity for utilizing body fat without ketosis by man within the range of temperatures studied.

The respiratory volume in most patients is large in comparison with the volume of oxygen removed. Under conditions of rest normal persons remove from 3 to 5 per cent of oxygen from inspired air, and this proportion does not change much in moderate activity. There is a tendency in these patients for this proportion to be reduced as the temperature falls. When the lower temperatures are reached, less than 2 per cent oxygen may be removed and the expired air may contain as little as 1.5 per cent CO_2. The significance of this alteration in respiratory regulation will be discussed in connection with observations on the properties of arterial blood.

Respiratory function of the blood. The changes to be expected in properties of the blood include, on the one hand, those that can be predicted from our knowledge of the physicochemical properties of the system and, on the other hand, those that depend on the physiological reactions to cold. In the first category are the changes in solubility of gases, the decrease in base-binding capacity of proteins and the increase in affinity of hemoglobin for oxygen. In the other category one may find a decrease in available base because certain anions accumulate, a change in hemoglobin concentration because of a redistribution of body fluids, and responses of pCO_2 and pH that are dependent on the foregoing factors as well as on the effect of cold per se on tissues involved in respiratory regulation.

The effect of temperature on the base-binding capacity of plasma proteins can be calculated from existing data. The equation at 38°, according to Van Slyke, Hastings, Hiller and Sendroy (6), is

$$BP_s = 0.104\ (P)_s\ (pH_s - 5.08) \qquad (1)$$

where BP_s = milliequivalents of base bound per liter of plasma
P_s = grams of protein per liter of plasma
0.104 = a factor measuring buffer value
5.08 = the pH of minimal base binding at 38°

The pH of minimal base binding, commonly referred to as pI, varies with temperature. The approximate relation for plasma protein, according to Stadie, Austin and Robinson (7) is

$$\frac{\Delta pI}{\Delta t} = -0.02$$
$$\text{or } \Delta pI = -0.02\ \Delta t$$

By substitution, the general equation taking temperature into account is

$$BP_s = 0.104\ P_s\ [pH_s - (5.08 + \Delta pI)]$$
$$\text{or } BP_s = 0.104\ P_s\ [pH_s - (5.08 - 0.02\ (t_0 - 38°))] \qquad (2)$$

where t_0 = observed temperature in °C.

Equation (2) has been used for calculating proteinate of serum in these experiments; these results are being published elsewhere.

In our studies of acid-base balance in the blood the CO_2 and O_2 contents are determined directly on the Van Slyke apparatus. Similar analyses are made of blood equilibrated at 37° and at a CO_2 tension of about 40 mm.Hg. In most instances, when the body temperature is reduced a second portion is equilibrated at body temperature; the CO_2 content of this gives the CO_2-combining capacity of the blood *in vivo*. With suitable correction for dissolved oxygen, one should obtain the same O_2 combining capacity in blood samples equilibrated at different temperatures, but the CO_2-combining capacity increases as the temperature decreases. This depends on the decreasing base-binding capacity of blood proteins—both of the plasma proteins, as described above, and of hemoglobin as well.

In view of the heterogeneity of the system, the effect of cold on the base-binding capacity of blood is too complicated to be described precisely in simple terms. An empirical relation has been worked out that holds with sufficient accuracy for such experiments as these. It is based both on such pairs of observations as those just described and also on two experiments in which normal blood was equilibrated with CO_2 tensions ranging from 10 to 80 mm. and at temperatures of 23, 30 and 37°. The empirical relation between T_{40} (the millimols of CO_2 in oxygenated

blood at a CO_2 tension of 40 mm.Hg), and the change of T_{40} per degree, is as follows:

T_{40} at 37°, *mM*	$\Delta T_{40}/\Delta t$
22	−0.45
20	−0.37
18	−0.29
16	−0.24
14	−0.21
12	−0.19
10	−0.17

Values for pH and for pCO_2 in samples of blood so analyzed and equilibrated can be calculated in the usual way, remembering the effects of temperature on pK′ and on the solubility of CO_2. The former relation, according to Cullen, Keeler and Robinson (8), is

$$\frac{\Delta pK'}{\Delta t} = -0.005$$

The solubility of CO_2 in serum, taking the value for 38° established by Van Slyke, Sendroy, Hastings and Neill (9) as a standard, can be calculated for other temperatures assuming that the effect of temperature on solubility is the same as in water and that the water content of serum is 940 cc. per liter. The calculated values are given in the following table. Factors for oxygen solubility in blood of normal hemoglobin concentration are given in the same table. These are based on the factor for normal blood at 38° established by Sendroy, Dillon and Van Slyke (10) and are calculated to other temperatures on the assumption that the solubility varies with temperature as in pure water.

TEMPERATURE	CO_2	O_2
°C.	*mM/l. serum*	*mM/l. blood*
25	0.0424 pCO_2	0.00163 pCO_2
26	0.0411	0.00161
27	0.0399	0.00158
28	0.0388	0.00155
29	0.0379	0.00153
30	0.0370	0.00150
31	0.0361	0.00148
32	0.0353	0.00146
33	0.0345	0.00144
34	0.0337	0.00143
35	0.0329	0.00141
36	0.0321	0.00139
37	0.0313	0.00138
38	0.0306	0.00136
39	0.0299	0.00135
40	0.0293	0.00133

Temperature affects both the base-binding capacity of hemoglobin and also, as described experimentally by Brown and Hill (11), its combination with oxygen. For the form of the curve at constant pH we have the data given by Dill, Edwards, Florkin and Campbell (12). They also give the change in position with pH_c. It is somewhat more convenient to have their results in terms of pH_s: this calculation we have made, using the relation between pH_s and pH_c given in an alignment chart by Dill, Edwards and Consolazio (13).

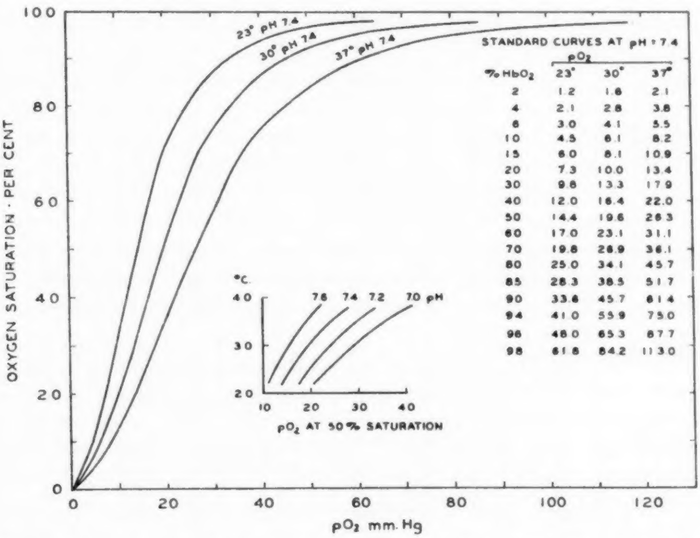

Fig. 1. Oxygen dissociation curves of human blood at a pH_s of 7.4 and at 23, 30 and 37°C. The inserted figure shows the relation of pO_2 at one-half saturation to temperature at four pH values. Since oxygen dissociation curves can be transformed from one position to another by multiplying abscissae by a constant factor, it is possible to construct a dissociation curve for any condition within the given limits of pH and temperature.

The results of the foregoing calculations yield the curves of figure 1. The three curves of figure 1 A define the combination of oxygen with hemoglobin at 23°, 30° and 37°C. Figure 1 B gives the relation between the position of the curves (pO_2 at one-half saturation) and four pH values within the same temperature range. Assuming that the form of the oxygen dissociation curve is independent of pH and temperature, these figures yield by interpolation the percentage saturation for any pO_2 and any pH_s if varied by CO_2 within the given limits or, if desired, a complete oxygen dissociation curve at any desired condition.

TABLE 3

Respiratory properties of the blood

SUBJECT	HOUR OF TREAT-MENT	BODY TEMPERATURE	HbO_2 CAPACITY	HbO_2 CONTENT	TOTAL CO_2	pH	pCO_2	T_{40} At body temperature	T_{40} At 37°
		°C.	mM/l.	per cent	mM/l.		mm. Hg	mM/l.	mM/l.
Mrs. H. M.	0	37.0	7.8	96.8	22.6	7.41	40.4	22.4	22.4
	8	30.0	9.5	99.2	15.4	7.37	28.8	17.8	16.1
Miss S. E.	0	37.3	7.4	99.1	23.3	7.35	48.0	21.7	21.8
	4	34.6	9.4	93.9	20.6	7.28	47.5	19.0	18.3
	20	24.2	10.8	93.0	20.0	7.22	45.2	19.0	16.0
	29	29.5	9.3	97.2	17.3	7.33	34.0	18.6	16.7
Mr. W. D.	0	37.0	8.8	100.0	20.8	7.37	41.0	20.4	20.4
	7	35.0	9.4	98.8	17.0	7.31	37.8	17.3	16.8
	21	26.7	9.9	100.0	15.1	7.38	26.7	18.3	15.8
	45	29.5	10.4	93.8	7.8	7.22	19.5	11.9	10.7
Miss S. J.	0	37.0	8.9	96.2	19.0	7.43	33.5	20.3	20.3
	9	35.1	9.0	96.0	19.1	7.33	40.4	19.0	18.4
	25	26.9	9.5	97.7	17.0	7.38	30.0	19.1	16.6
Miss B. E.	10	34.1	8.1	93.7	21.1	7.31	44.2	20.1	19.1
	21	31.6	8.1	96.7	17.9	7.41	30.0	20.0	18.3
	29	33.4	7.1	97.2	19.0	7.35	37.0	19.4	18.3
Mr. S. R.	0	37.0	9.0	95.5	21.7	7.39	41.8	21.2	21.2
	9	36.3	9.7	95.7	20.5	7.26	51.5	18.3	18.1
	22	36.1	10.0	97.3	15.3	7.23	41.2	14.9	14.7
	31	38.5	9.2	95.7	16.3	7.21	44.0	15.5	15.8
Miss H. I.	9	32.2	8.2	92.4	18.5	7.28	40.6	18.3	17.0
	21	31.2	7.7	92.1	18.9	7.33	36.2	19.6	17.9
	38	38.3	6.6	95.6	19.6	7.24	49.8	18.0	18.3
Miss P. M.	0	37.0	6.9	95.7	22.0	7.38	41.2	21.5	21.5
	21	32.1	7.1	100.0	19.1	7.38	33.5	20.2	18.6
	34	37.7	6.4	96.0	16.7	7.35	33.8	17.8	18.0
Miss M. F.	0	37.0	7.5	97.2	21.1	7.39	39.2	21.1	21.1
	23	33.0	9.2	96.0	16.4	7.33	33.8	17.6	16.6
	32	40.0	6.8	91.5	17.6	7.33	51.8	15.1	15.8

A simple application of the foregoing principles has been made. The observations on percentage saturation (table 3) have been segregated into three temperature ranges: 35° or above, 30° or below, and intermediate

values. The averages, together with the estimated pO_2 values, are as follows:

NUMBER OF CASES	MEAN TEMPERATURE	MEAN SATURATION	MEAN pH_s	pO_2
	°C.	*per cent*		*mm. Hg*
15	37.1	96.3	7.35	98
8	32.8	95.3	7.33	74
6	27.8	96.8	7.33	70

While we have no analyses of alveolar air, the fact that the ventilation was increased out of proportion to the increase in oxygen consumption implies that the alveolar pO_2 was greater than usual. Despite hyperventilation, the pO_2 of arterial blood declines with decreasing body temperature. This is not what one might predict, but there are several factors which may have contributed to this result. The slowing-down of the various chemical reactions in the blood which are concerned with the uptake of oxygen in the lungs may be a contributing factor, but it is probable that reduction in the rate of diffusion through the lung membrane is the most important cause. In one patient edema was evident, and it may be that a slight accumulation of fluid in the alveoli may occur at subnormal body temperatures.

It is notable that almost without exception the pH is on the acid side and the CO_2-combining capacity, even when measured at body temperature, is reduced. The state is one of acidosis partially but not wholly compensated for by hyperventilation. One patient (Mr. W. C.) experienced about twice as great a reduction in CO_2-combining capacity as any other and was also one of the most acidotic. He was also one of the most combative.

While the acid-base balance of the blood probably is chiefly responsible for the respiratory regulation in these patients, it is possible that the oxygen tension in arterial blood is reduced enough to stimulate respiration through the mediation of the carotid body. Reference may be made to the recent work of Asmussen and Chiodi (14) describing the rôle of the arterial pO_2 in regulating respiration.

The distribution of body water and the exchange of fluids will be discussed elsewhere and are mentioned here because of the considerable fluctuations observed in serum protein concentration and in the proportion of hemoglobin. In the early experiments little fluid was supplied and the hemoglobin increased, in the case of Miss S. E. by one-half. In most of the later cases the hemoglobin remained more nearly normal, but it decreased by one-third in Miss H. I.

Discussion. Our observations on the metabolic rate in hypothermia

do not agree well with those reported by Smith and Fay (2). Using an indirect method, they commonly found a reduction ranging from −6 to −25. Our results are what one would expect from the numerous measurements made by Benedict and Lee (1) on the marmot as it enters hibernation. Only when shivering moderates should one expect a reduction in metabolic rate. It is interesting in this connection to speculate on the degree to which processes of homeostasis are thrown off balance when the body temperature is so greatly reduced. While shivering may demand more than twice the basal energy exchange, chemical processes, notably those of an enzymatic nature, are greatly slowed. This field deserves further exploration.

It is of interest to collate our observations on man at low body temperatures with those described in Walther's classic *Beiträge zur Lehre von der thierschen Wärme* (15). Walther observed that in rabbits killed while at a temperature of 20° the lungs were filled with blood and a watery serous exudate extended through the parenchyma and bronchioles. This fits in with our observation that the pO_2 in arterial blood is reduced: conditions for diffusion may be unfavorable because of pulmonary edema.

Another observation of Walther's may be interpreted similarly. While rabbits at 20° do not shiver and will die if left in a cool place, they can be revived, with slowly rising body temperature, by means of artificial ventilation, even though the air temperature may be 10 to 12°. (Walther claimed that this was the first proof that animal heat depends on the entrance of air into the lungs.) It may be that the arterial oxygen pressure is so greatly reduced by pulmonary edema that vital processes, including shivering, sink to lower and lower levels until they are terminated. Artificial ventilation raises the oxygen partial pressure enough to swing the balance in the other direction. In this connection Nielsen and others (16) have shown that shivering is inhibited by anoxia, even at ordinary body temperatures. Since it is also reduced in intensity by low temperature, it seems likely that a combination of cold and anoxia may produce a complete cessation of shivering and in effect a poikilothermous animal. An additional possibility is that the alkalosis of artificial ventilation may contribute to the result that so surprised Walther.

In general, any state that handicaps oxidative processes in the nervous system inhibits shivering. Besides cold, anesthesia and anoxia, mention should be made of the discovery by Dworkin and Finney (17) that shivering is inhibited in insulin hypoglycemia. Possibly in our experiments shivering abates not entirely because of falling temperature but in part because depletion of carbohydrate reserves lowers the blood sugar significantly. This question is being investigated.

Walther was the first to point out that cold produces a state of anesthesia

in which animals are rendered suitable for operative procedures. Muscles remain irritable while the reduction in peripheral blood flow lessens hemorrhage. Parker (18) has commented on this phenomenon.

Numerous observations like those of Walther prove that most mammals are apt to die if their temperature falls below 20° and almost certain to die before 10° is reached. Yet hibernating animals can survive a temperature of 2 or 3°C. Does this imply the possession of body constituents of unusual physicochemical properties? So far as we can find, Tait was the first to suggest that their lipoids may have unusually low solidification

TABLE 4

Solidifying points of body fats

SPECIES	AUTHOR	SOLIDIFYING TEMPERATURE
		°C.
A. Hibernating mammals		
Hedgehog (*Erinaceus europaeus*)	Pawletta (20)	+3 to +5
Marmot (*Arctomys sp*)	Pritzker and Jungkunz (21)	−15
Skunk (*Mephitis sp*)	Pawletta (20)	+24
Bear (*Ursus torquatus*)	Ueno and Kuzei (22)	30*
B. Nonhibernating mammals		
Badger (*Meles taxus*)	Lewkowitsch (23)	17–19
Hare (*Lepus timidus*)	Lewkowitsch (23)	17–30
Rabbit (*Lepus cuniculus*)	Lewkowitsch (23)	17–30
Sheep	Lewkowitsch (23)	36–41
Ox	Lewkowitsch (23)	35–37
Dog	Lewkowitsch (23)	21–23
Cat	Lewkowitsch (23)	24–26
Hog	Lewkowitsch (23)	27–30
Man	Lewkowitsch (23)	15

* Melting point.

temperatures (19). This hypothesis was proposed after he had demonstrated that, while the excised heart of a hibernating animal will continue to beat at 0°, that of nonhibernating animals ceases to beat at 17°.

Data presented in table 4 bear out Tait's suggestion. The two animals on which observations have been made and which are known to hibernate throughout the winter have fats that remain fluid at low temperatures. The skunk and the bear are often active in midwinter and are therefore in a special category. Furthermore, the recorded solidifying temperature of skunk fat, 24°, does not correspond to the observation (24) that much of the fat is liquid at ordinary temperatures, separating into two phases as it is cooled. The data in table 4 must be considered as approximations, since

the solidification temperature cannot be determined precisely and certainly varies with diet, if not also with season. The characteristics of fats from different parts of the body are also known to vary.

The picture of an inadequately clothed man lost in a blizzard is illuminated by our findings. So long as he continues to walk, production of heat may balance its loss. Eventually exhaustion not only forces him to stop but may also, mediated by low blood sugar, keep shivering at a low level. As his temperature falls, shivering becomes less intense and eventually coma and death ensue.

Finally, we may say that hypothermia is a state of light anesthesia in which certain functions, such as those concerned with temperature regulation and acid-base balance, remain moderately effective. While the total metabolic exchange is apt to be above the basal level, this does not exclude the possibility that such organs as the brain, the liver and the kidney decline in activity with decreasing temperature. Studies are being continued in this direction.

SUMMARY

The total energy exchange throughout long periods of subnormal body temperature may remain above the basal level and may be two or even three times the basal level. This depends on shivering, voluntary activity, and a muscular rigidity of unknown origin.

The effects of temperature change on the blood as a physicochemical system have been described. The observed effects included those that could be predicted from our knowledge of the blood and others that reflect physiological responses to cold.

There is a trend toward acidosis, evidenced by a reduction commonly of one-quarter, in one case of one-half, in alkaline reserve. This reduction, measured at body temperature, occurs despite the fact that *in vitro* alkaline reserve increases with decreasing temperatures.

Hyperventilation provides additional evidence of decreasing alkaline reserve. Expired air may contain less than 1.5 per cent CO_2 when the arterial pCO_2 is only slightly reduced. Despite the hyperventilation and the presumed increase in alveolar pO_2, the arterial pO_2 decreases. These facts point to poorer diffusion in the lungs, a deduction supported by Walther's observation of pulmonary edema in hypothermic rabbits. Within the range of our observations, the shift in the oxygen dissociation curves to the left was enough to maintain a normal arterial saturation with oxygen.

Respiratory regulation remains effective even at 25°C. Not only the acid-base balance but perhaps also the arterial pO_2 is concerned in this function.

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IS HISTAMINE ABLE TO MAINTAIN AN AUGMENTED PEPSIN RESPONSE COMPARABLE TO THAT OF PILOCARPINE?

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Up to the present time, the data relative to the effect of histamine on pepsin secretion have been obtained by the use of the single or double histamine test. Evidence has been presented (1) showing that the single or double histamine test yields data which have no value in critically determining whether the histamine produces an increased pepsin output by stimulation (2) or by a "washing-out" process (3, 4). It appeared to us that the "continuous histamine" test (5) would provide an ideal method of approach because a brisk continuous uniform secretion would insure against accumulation and "washing-out" processes, as well as against deleterious changes in the activity of pepsin due to wide variations of the pH of the gastric juice.

Accordingly, in this study we have used the continuous histamine test to determine whether relatively large doses of histamine given every 10 minutes will evoke and maintain in the vagotomized total stomach pouch of dogs a larger pepsin output than small doses of histamine. The study has been extended to ascertain how the pepsin output under "continuous histamine" compares with that of pilocarpine, a drug which every one agrees stimulates pepsin secretion (6).

Experimental procedure. Five dogs with vagotomized total-stomach pouches and thoroughly accustomed to experimentation were the subjects. All experiments were started after an over-night fast. The dose of histamine was adjusted to each animal so that in any series of tests, the volume-rate responses of the various animals were similar. No attempt was made in the pilocarpine series, however, to achieve such uniformity. The dose of pilocarpine was adjusted to the animal on the basis of 0.20 mgm. per 10 kilo body weight. The animals received a given dose of either or both drugs every 10 minutes throughout the experiment (see table 1). It was sometimes necessary to shift the histamine dose during an experiment to keep the volume-output within the desirable range. There was no consistency about the direction of the shifts or the time at which they occurred. The amount of the change in dose rarely exceeded

0.010 mgm. Only two changes were made necessary in the pilocarpine dosage (see footnote, table 1).

All gastric drainage for the first 40 to 60 minutes was discarded. By this time the secretory response to histamine had become steady, and that

TABLE 1

The dosage, in milligrams, of histamine and pilocarpine given subcutaneously every 10 minutes

ANIMAL	HISTAMINE		PILOCARPINE SERIES (c)
	Low series (a)	High series (b)	
	mgm.	*mgm.*	*mgm.*
C	0.020	0.080	0.20
M	0.025	0.100	0.34
F	0.025	0.090	0.10*
H	0.030	0.100	0.22
B	0.065	0.120	0.20*

* The dose for dog F had to be reduced from 0.21 mgm. pilocarpine to 0.10 mgm. during the first hour because of hemorrhage into the pouch. Secretion failed during the fifth hour in dog B, and increasing the dose to 0.34 mgm. pilocarpine temporarily revived it.

TABLE 2

Key to the experiments performed

SERIES NAME	DURATION	NUMBER AND LENGTH OF PERIODS	DRUG AND DOSE (FROM TABLE 1)	NUMBER OF EXPERIMENTS
Low histamine	8 hrs.	(1)—8 hrs.	(a)	11
High histamine	8 hrs.	(1)—8 hrs.	(b)	10
Low-high-low histamine	7 hrs. and 20 min.	3		11
		P* (1) = 2 hrs.	(a)	
		P (2) = 2 hrs., 40 min.	(b)	
		P (3) = 2 hrs., 40 min.	(a)	
Pilocarpine	6 hrs.	(1)—6 hrs.	(c)	5
Histamine-pilocarpine	7 hrs. and 20 min.	3		5
		P (1) = 2 hrs.	(a)	
		P (2) = 2 hrs., 40 min.	(a) plus (c)	
		P (3) = 2 hrs., 40 min.	(a)	

* The "P" indicates "period." The extra 40 minutes were allowed for transition from one secretory rate to the other.

to pilocarpine had become sufficient to yield adequate samples. The collection interval was 20 minutes. All samples were analyzed for free and total acidity, and for pepsin by the hemoglobin method (1). A Coleman glass electrode was used to determine pH in the low-high-low histamine series. A key summarizing the important features of the 5

series of experiments is presented in table 2. In each series the same five animals were used.

The animals were encouraged to drink water during the experiments. In half of the experiments in the high histamine series, representing one on each animal, additional fluid and chloride was supplied by 400 to 600 cc. physiological saline given subcutaneously during the second to fifth

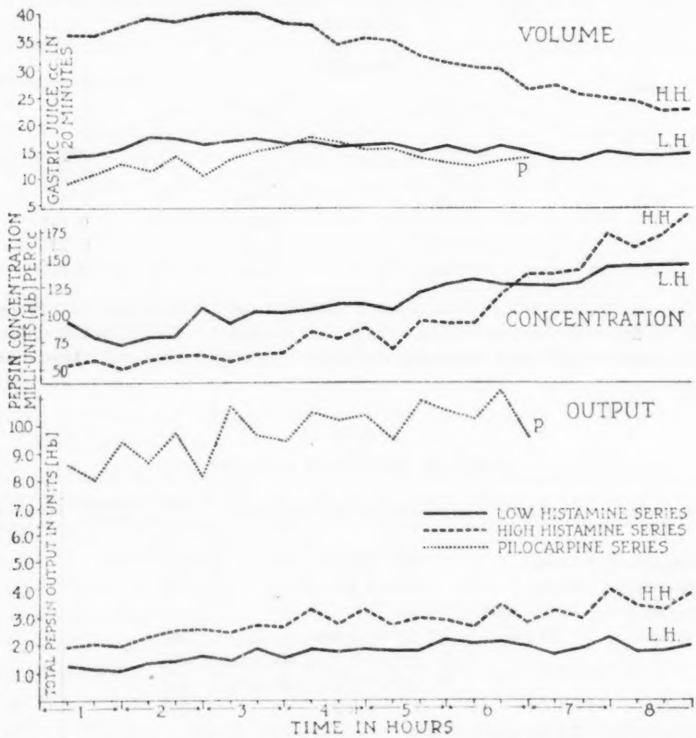

Fig. 1. Curves representing the arithmetical average of volume responses, pepsin concentration, and pepsin output in the low continuous histamine series, the high continuous histamine series, and in the continuous pilocarpine series.

hours. It was hoped that this measure would prevent the gradual decline in secretory rate which generally set in about the 4th hour in this series. This procedure tended to delay the onset of the decline but did not prevent it.

The observational unit employed in the graphs and in the statistical analysis has been the 20-minute outputs of volume and pepsin. The statistical methods used are the same as those employed previously (1).

Data and discussion. *The high and the low histamine series.* Results of the high and low histamine series are presented as mean curves of arithmetical averages in figure 1. It is evident that the larger doses of histamine in the high series, in contrast to the low series, produced twice as much gastric juice, possessing a distinctly *lower pepsin concentration but a higher total pepsin output.* This generalization is confirmed by statistical analysis of the 237 pairs of samples collected in the two series, and the data of 4 of the 5 animals when examined individually bear this out (table 3). In one animal, dog B, the reversed response was observed. This dog was unusually refractory to histamine during the time the low series tests were performed on him; he required over twice as much histamine as the other animals (table 1). The reversed response of this one animal is completely lost when the data are statistically analyzed as a whole. We may now

TABLE 3

Summary of pepsin-output in the high and low histamine series

ANIMAL	CORRELATION	PAIRS OF SAMPLES	MEAN MINUTE-OUTPUT OF PEPSIN, MILLI-UNITS (Hb)		SIGMA OF DIFFERENCE	CRITICAL RATIO
			High series	Low series		
C	+0.77	48	154 ±14.0	84 ±7.5	9.6	7.3
M	+0.16	48	124 ± 4.5	81 ±3.2	5.1	8.1
F	+0.45	45	234 ±14.5	155 ±9.6	13.4	5.8
H	+0.33	48	142 ± 5.0	55 ±3.3	5.0	17.4
B*	+0.72	48	45 ± 3.5	67 ±4.6	3.1	7.0*
All dogs	+0.62	237	140 ± 5.1	87 ±3.5	4.7	11.1

* Dog B was found to be significantly reversed.

conclude without equivocation that the *pepsin liberating mechanism*, as a rule, responds to histamine by an increased output of pepsin.

A second observation of importance is that in both series the pepsin concentration and pepsin output showed an unmistakable tendency to increase over the 8-hour period. This is particularly true of the high histamine series. The definitely significant climb during the last 3 hours of the high series, occurring in spite of a steadily diminishing volume-output, is especially significant when viewed in relation to the work of Bowie and Vineberg (7). These workers, using dogs, under chloralose and urethane anesthesia and hourly doses of histamine (2 mgm. per 10 kilo body weight for 8 hours) found that the pepsin output which had been low from the beginning fell to zero at the end of the fourth hour. The use of chloralose, which has been reported to inhibit the gastric secretory response to histamine, may be responsible for these results (8). Lim and Liu (9), using the same hourly dose of histamine in Pavlov pouch dogs found that pepsin continued to be produced when the experiment was

continued as long as 24 hours, regardless of whether food and water were withheld during this time or not. The data of these latter workers also show that the average hourly outputs of pepsin tend to increase to a maximum about the 8th hour, after which they fall off somewhat to remain fairly uniform to the end of the 24-hour observation period.

In view of the belief that the parietal cell is non-fatigable (10), it is interesting to note that, in spite of fluid and chloride replacements when fairly intensive histamine stimulation is maintained, as in the high histamine series, the parietal cells manifest a "partial fatigue," so named by Lim and Liu (9), whereas the chief cells apparently do not. This is not manifest when less intensive stimulation is similarly prolonged, as in the low histamine series.

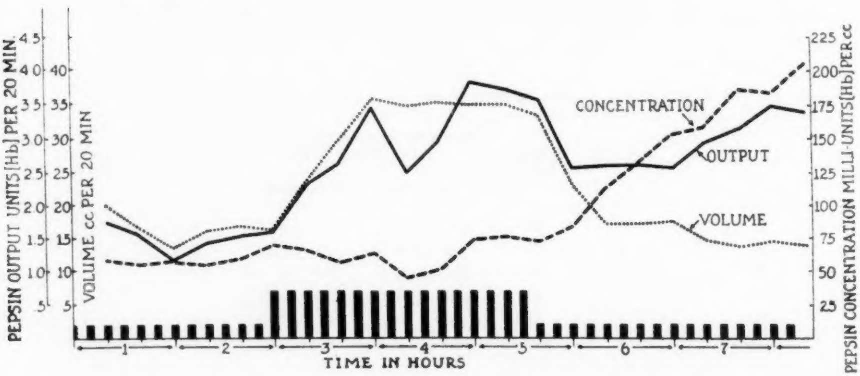

Fig. 2. Curves of the arithmetical averages of the responses in volume, pepsin concentration, and pepsin output in the low-high-low continuous histamine series.

Low-high-low histamine series. The ability of histamine in larger doses to evoke a significantly increased pepsin output is confirmed in the low-high-low histamine series of experiments (fig. 2). Excluding for the moment period 3 (fig. 2), the volume and pepsin outputs obtained in the first 2 periods are just what would be expected on the basis of the responses in the high and low histamine series shown in figure 1. The average outputs for the three periods are shown in table 4 and are compared with those of the low and high histamine series. The similarity of results is striking, except for the increased pepsin output during period 3.

The persistence of the augmented pepsin output in period 3, after the volume output had become adjusted to the lower histamine dosage, is an indication of an aspect of the pepsin secretory mechanism, as regards histamine, that has not been observed before, and which challenges explanation. It is perhaps an expression of the tendency of the pepsin out-

put to climb during continued uniform stimulation as observed in the high and low histamine series (fig. 1). We have at this time no explanation to offer for the sustained high peptic output after the histamine had been reduced and the volume response diminished by almost half, except to suggest that it is most likely due to an accumulative effect of histamine.

Comment. In the light of our observations, there is no evidence to support the view that histamine inhibits pepsin secretion (11). Neither do our data lend support to the view (3, 4) that the increase in pepsin output after histamine is due to a "washing-out" of previously secreted pepsin from the gastric tubules, for the continuous uniform secretion precluded accumulation of pepsin. Should it be suggested that temporarily increasing the histamine dosage increases the output of pepsin in the low-high-low histamine series, because more gastric glands were caused to secrete acid and hence more tubules were flushed out, we have merely

TABLE 4

Summary of mean minute-outputs of high histamine series, low histamine series and the low-high-low histamine series

NAME OF EXPERIMENT	VOLUME-OUTPUT	PEPSIN-OUTPUT
	cc.	*Milli-units (Hb)*
Low histamine series	0.79	87.0
High histamine series	1.65	144.0
Low-high-low histamine series		
Period 1	0.72	83.0
Period 2	1.67	158.7
Period 3	0.72	126.3

to observe how incompatible such a suggestion would be with the data in figure 2, especially period 3, wherein the output of pepsin was greater than at the start of the experiment when the same low histamine dose and volume response prevailed.

Whether the increased pepsin output after the injection of histamine is due to an actual direct stimulation of the chief cells by histamine cannot be absolutely decided by our data. The increased pepsin output rather than being due to a direct action of histamine on the chief cells might be due to the stimulation of the chief cells by the contact of acid with that portion of their surface exposed to the lumen of the glands. However, this is not likely since on resumption of low histamine dosage after the high histamine dosage there resulted a marked decrease in acid output without a proportional decrease in pepsin output. Unless one assumes that stimulation of the chief cells by acid contact continues after the flow of acid has markedly declined, it is difficult to avoid the conclusion that histamine directly stimulates the chief cells to secrete pepsin.

The pilocarpine series and the histamine-pilocarpine series. The question now arises in regard to how the increased pepsin output caused by histamine compares with that obtained with a substance such as pilocarpine, which is admitted to act as a stimulant of the pepsin secreting cells. A glance at figure 1 will convincingly show that when approximately equal volume responses are compared, the pilocarpine juice has about 5 times as much pepsin as the histamine juice. Moreover, when pilocarpine was administered in addition to histamine (fig. 3), the pepsin response was only as great as if the pilocarpine had been given alone, while the volume

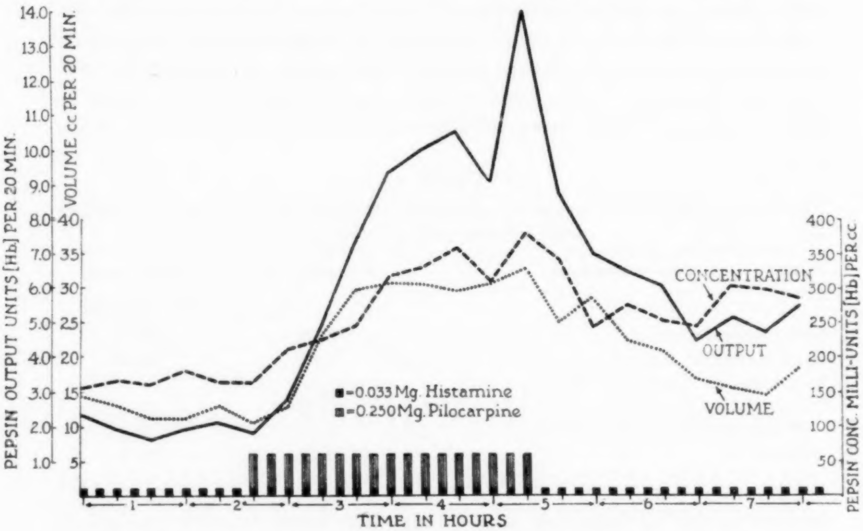

Fig. 3. Curves representing the arithmetical averages of volume, pepsin concentration, and pepsin output in the histamine-pilocarpine series.

responses showed an almost perfect summation of effect. This is especially evident from the totaled outputs shown in table 5.

As in the case of "continuous histamine," the tendency for the pepsin output to climb during the course of the continuous pilocarpine experiment is evident (fig. 1). The average minute-output for the last 4 hours of the pilocarpine series is 508.0 milli-units of pepsin, exactly the value achieved when the same amount of pilocarpine was administered with histamine, which alone was maintaining a pepsin output of 98.0 milli-units per minute.

When the pilocarpine was discontinued, there was an immediate and sudden fall in the pepsin output to a constant level which was almost three times as great as during the first period under the same degree of histamine stimulation. The suggestion of Necheles *et al.* (14) and Ihre (3)

that histamine and pilocarpine are synergistic as long as given together and that a temporary exhaustion response follows the cessation of either of the drugs is not sustained by this evidence. It should be pointed out, however, that a dosage factor may be involved in the question of synergism. After cessation of the pilocarpine, the volume response returned to the precise level under histamine alone, and the pepsin output, though less than in period 2, was markedly increased over histamine alone (figs. 1 and 2). If the tendency for pepsin output to continue at a high level after discontinuance of pilocarpine is interpreted as due to a continuance of the stimulating action of the drug, then the same interpretation may be applied to the same tendency manifested by histamine.

Evidence from experiments on humans has been reported (12, 13) which indicates that histamine is neither inferior nor superior to pilocarpine

TABLE 5

Summary of the mean minute-outputs of volume and pepsin in the pilocarpine series and in the histamine-pilocarpine-histamine series

NAME OF SERIES	VOLUME-OUTPUT	PEPSIN-OUTPUT	TYPE OF STIMULATION
	cc.	*Milli-units (Hb)*	
Pilocarpine series	0.68	475.0	Pilocarpine (c)
Hist.-Hist.P.-Hist.			
Period 1	0.82	98.0	Low histamine (a)
Period 2	1.54	507.0	Low histamine (a) plus pilocarpine (c)
Period 3	0.90	273.0	Low histamine (a)

in evoking gastric secretion as measured by volume, acidity and pepsin output. Our results on the dog do not permit us to agree with such a view. Although a species difference may play some part in this discrepancy, we believe that their use of the double histamine test and its inherent errors (1) is the most responsible factor.

SUMMARY. 1. Pepsin output is not inhibited or diminished during the course of an eight-hour "continuous histamine" test (a small dose of histamine subcutaneously every 10 min.) in dogs with pouches of the entire stomach and the vagal innervation cut. Indeed, it tends to increase with time especially when higher dosages of histamine are used.

2. The minute-output of pepsin in response to large doses of histamine such as 0.10 mgm. given every 10 minutes is at all times significantly larger than that in response to smaller doses such as 0.025 mgm. given every 10 minutes.

3. If during the course of a submaximal "continuous histamine" test (0.025 mgm. every 10 min.) the histamine dose is increased (0.10 mgm. histamine every 10 min.), the pepsin output will increase.

4. If during the course of a submaximal "continuous histamine" test the dose is increased for a period and then decreased again to the submaximal level, the output of pepsin continues at a higher level than during the first submaximal dosage period; i.e., the effect of the higher dose of histamine in increasing pepsin output tends to continue after resumption of a lower dose of histamine.

5. Pilocarpine given subcutaneously every 10 minutes in doses which produce a volume-rate response comparable to a low dose of histamine yields a pepsin output five times that of the histamine. The output, as with histamine, tends to increase during the course of a 6-hour experiment.

6. When pilocarpine and histamine are given together a summation of the effect of the two drugs is observed in the volume output, but the pepsin output is typical of pilocarpine when administered alone. The usual effect of histamine, which was continued after cessation of the pilocarpine, was unchanged, but the pilocarpine effect on pepsin output continued as in the case of histamine (see conclusion 4).

CONCLUSION

Under the conditions of our experiments, if pilocarpine is considered to stimulate directly the pepsin secreting cells, histamine does likewise on the basis of similar evidence and analogous deductions. The only difference is that with similar volume outputs of juice, pilocarpine yields considerably more pepsin than does histamine.

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STUDIES ON THE DISTRIBUTION OF RADIOACTIVE FLUORIDE IN THE BONES AND TEETH OF EXPERIMENTAL ANIMALS[1]

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Investigations of various skeletal abnormalities including chronic fluorosis (De Eds, 1933; Peirce, 1939) dental caries (Cox, 1940; Hodge, 1940) and rickets (Morgareidge and Finn, 1940) all point to a significant relationship between fluoride and the inorganic metabolism of bones and teeth. One of the great difficulties in fluoride studies has been the exacting methods necessary for the chemical determination of fluoride in the calcified tissue. Recently the radioactive isotope F^{18} has been used for in vitro mineral metabolism studies (Volker, Hodge, Wilson and VanVoorhis, 1940). To date in vivo researches using the same agent have not been attempted. Since it seemed probable that such an experiment might increase our knowledge of the biochemistry of fluorides, a series of experiments with the radioactive isotope was attempted.

PROCEDURE. Radioactive fluorine was obtained from the Department of Physics of this University through the courtesy of Dr. S. N. VanVoorhis. The isotope was prepared by bombarding the oxygen in water molecules by the proton beam of the cyclotron. The nuclear reaction is as follows:

$$\overset{18}{\underset{8}{0}} + \overset{1}{\underset{1}{H}} \longrightarrow \overset{18}{\underset{9}{F}} + \overset{1}{\underset{0}{n}}$$

The unstable isotope has a half life of 112 minutes and reverts to O^{18} by the emission of a positron as follows:

$$\overset{18}{\underset{9}{F}} \longrightarrow \overset{18}{\underset{8}{0}} + \overset{0}{\underset{1}{e}}$$

Two groups of experimental animals were used in this study. The first group was composed of 5 adult rats, weighing approximately 250 grams

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each, and the second group 4 adult cats weighing from 2.6 to 4.0 kgm. Rats 4 and 5 were suffering from chronic fluorosis induced by the long time ingestion of a diet containing approximately 300 p.p.m. of fluoride. Animals in the first group were given intraperitoneal injection of 2 to 4 mgm. of fluorine as sodium fluoride in physiological saline plus a known amount of marked fluoride. Thirty-five minutes after the administration of the fluoride, the rats were sacrificed by decapitation and a sample of blood collected and oxalated. Samples of the molar crown, femur shaft, inferior and superior maxillae, incisor tips, and whole incisor minus the incisor tip were cleansed of the adhering tissue, broken up, weighed and dissolved in 6 N/HCl. An attempt was made to remove the pulp tissue from the molar crowns without disturbing the coronal dentin. Two cubic centimeter aliquots of the dissolved tissues were placed in a counting cup of the Geiger-Müller scale-of-4 counter and the number of counts determined for duplicate or triplicate five-minute periods. At suitable intervals the radioactivity of the standard radioactive fluoride solution was determined, as well as the background count and the counter sensitivity.

Animals in the second group were given intravenous injections of 10 mgm. of labeled fluoride in physiological saline solution. The submaxillary saliva was collected from the cannulated duct, the salivary flow being increased by electrical stimulation of the chorda tympani nerve. Periodic blood samples were also taken. Both these phases of the study were carried out by J. H. Wills of the Department of Physiology and are reported by him in detail elsewhere (Wills, 1940). After the collection of the last blood sample, the animal was immediately sacrificed and samples of whole teeth, depulped cuspid crowns, superior and inferior maxillae, femur shaft, intestinal washings, and salivary glands were collected. In order to make certain the complete removal of the residual pulp tissue from the cuspid crowns, the walls of the pulp chambers were cleaned with a dental burr. The calcified tissues were prepared for counting as previously described. The soft tissues were ground in a mortar with sand and extracted with saline solution. In each of the four cats the intact bladder was removed and aliquots of urine taken. In no case did urination occur during the experimental period. Radio fluoride determinations were made by the same procedure as used for the group 1 samples.

Findings. The results obtained with the group 1 rats may be seen in table 1. It will be noted that the concentration of the fluoride in the blood at the time of death shows marked variation. Since the blood level of the fluoride is probably directly dependent on the rate of absorption from the gut, it would appear that this property is quite variable in rats when the fluoride is administered by intraperitoneal injection. These variations are most likely to be found in short-term experiments such as those necessitated by problems involving short half-life isotopes. This same factor,

the blood level of the fluoride, undoubtedly influences the rate and percentage of absorption by the various calcified tissues. The percentage of the total dose per gram of tissue figures indicate that the calcified tissues take up fluoride from the circulating blood; the amount of fluoride in the skeletal tissues is roughly parallel to the adequacy of the blood supply with no detectable amounts of fluoride being present in the incisor tip. The figures for the incisor minus the incisor tip, are slightly below those observed for whole bone, but since these samples contain at least half the total amount as fully calcified and erupted tooth substances, the percentage of total dose per gram of tissue of the remaining half (actively calcifying root) is probably in excess of that observed for jaw bone or femur. This would be expected since, in addition to the possibility of a

TABLE 1

Radioactive F distribution in the rat

RAT NUMBER	DOSE OF FLUORIDE	PER CENT OF TOTAL DOSE PER GRAM OF TISSUE				
		Blood	Molar crown	Femur shaft	Combined maxillae	Incisor minus incisal third
	mgm.					
1	4	0.128	0.294	0.371		
2	4	0.370	0.435	0.647		
3	2	0.208	0.234	1.327	1.227	1.197
4*	2	0.128	0.229	0.901	0.993	0.786
5*	2	0.170	0.421	0.716	0.910	0.867

* Fluorosed.

Incisor tip *gave* no count except in rat 2 where a portion of the middle third was included.

Salivary gland rat 2 (800 mgm.) gave 0.098 per cent of total dose per gram of tissue.

normal exchange reaction, the process of calcification is proceeding at an accelerated rate in this area. The presence of detectable amounts of fluoride in the cleansed molar crowns was a consistent finding and probably indicates that fluoride is being deposited in that part of the dentin approximating the pulpal tissue.

No striking difference in F metabolism was evident in a comparison of the normal and fluorosed animals. The results obtained with group 2 cats may be seen in table 2. Cats 1 and 2, which were allowed to live for approximately two hours following the administration of fluoride, may be compared with cats 3 and 4 which were sacrificed after a wait of approximately 30 minutes. It will be noted that the blood fluoride level in cats 1 and 2 was much less than that of the calcified tissue, whereas in cats 3 and 4 the reverse was true. This would seem to indicate that the injected fluoride rapidly leaves the circulating blood and becomes deposited in the

skeletal tissues. As previously noted in the group 1 experiments, the percent of total dose radio fluoride per gram tissue varies with the extent of contact of decalcified tissue with the circulating blood, being greatest in the bone and negligible in the depulped cuspid crowns. The differences observed in the two types of bone, namely, the femur and mixed jaw bone samples, may be due in part to their anatomical dissimilarity, the maxillae being flat or skull bones and the femur a long bone. Also it is conceivable that the rate of fluoride deposition may follow a different curve for various bones, depending on the adequacy of the intraosseous circulation. The failure to demonstrate a similar difference between the rat maxillae and femur shaft needs further investigation. Two possible factors may be cited in this connection. First, a comparatively greater portion of the

TABLE 2

Radioactive F distribution in the cat

CAT NUMBER	WEIGHT	PER CENT OF TOTAL DOSE OF FLUORIDE PER GRAM OF TISSUE				PER CENT TOTAL DOSE F IN SALIVA	PER CENT TOTAL DOSE F IN URINE
		Blood	Whole tooth	Combined maxillae	Long bone		
	kgm.						
1	2.8	0.015	0.030	0.095		0.1102	14.4
2	2.6	0.016	0.032	0.101	0.037	0.0415	14.6
3	4.0	0.060	0.014	0.030		0.0877	22.7
4	3.5	0.064	0.015	0.038	0.020	0.0540	10.4

Each cat received 10 mgm. of NaF plus the radioactive isotope.

Samples of intestine, salivary gland and cuspid tooth crowns contained insufficient fluoride for estimation.

Time of salivary fluoride secretion in cats 1, 2, 3, 4 was 117, 106, 19 and 21 minutes respectively.

rat femur was taken for analysis than was used in the cat experiments. This was necessitated by the relatively small size of the rat's femur. Second, a greater dose of fluoride per gram of body weight was given to the rats than was administered to the cats.

The percentage of the total doses of fluoride secreted in the saliva was calculated from the data of Wills. These figures and those obtained for the percentage of the total dose excreted in the urine, are approximately the same for the one-half and the two hour animals, indicating that the greater proportion of the salivary and urinary fluoride was excreted within the first 30 minutes of the experimental period, when the blood level of fluoride is correspondingly high.

Discussion. It will be noted that the actively calcifying portions of the rat incisors show the greatest deposition of fluoride. This is in agreement with the observation that the incisors of the rat are extremely susceptible

to disturbances in calcification and pigmentation, as seen in chronic fluorosis, and is also in keeping with the chemical finding that the fluoride content of the rat incisor can be markedly increased on a high fluoride diet (Hodge, Luce-Clausen and Brown, 1939).

The high fluoride deposition in the bony skeleton is probably significantly related to the observation that the addition of fluoride to a rachitogenic diet decreases the severity of experimental rickets (Morgareidge and Finn, 1940).

The small fluoride secretion in the saliva and the lack of appreciable fluoride deposition in the incisal third of the rat incisor and depulped cuspid crowns may help to clarify the mechanism by which fluoride reduces caries incidence. In this connection we have found that the average fluoride secretion in the saliva of the group 2 animals totaled only one one-thousandth of the injected dose. Since the two-hour excretion approximated that of the half-hour excretion, it would appear that comparatively little fluoride was secreted in the saliva after the first half-hour. It should be remembered that the injected dose of fluoride (10 mgm.) is probably in excess of the amount found in the daily diet of humans, and that the experimental animals used were approximately $\frac{1}{20}$ the size of an average adult human male. The failure to find significant amounts of the labeled fluoride in the dental tissues well removed from the tooth pulp, does not support the possibility that fluorine may be deposited via the circulation in the dental enamel. This point is of particular interest to workers in caries research, since it has been shown that the fluoride content of non-carious enamel is significantly higher than that of carious enamel, although no such difference exists between carious and non-carious dentin (Armstrong and Brekhus, 1938). However, the possibility still exists that the ingested fluoride is first stored in the bones and may later be released to the circulation where it could conceivably be deposited in the more inert portion of the teeth. Unfortunately, the short half-life of the radioactive isotope will not permit studies to test that possibility.

SUMMARY

Radioactive fluorine was given by intraperitoneal injection to five rats and by intravenous injection to 4 cats. Concentration of the blood fluoride fell rapidly with a corresponding rise in the calcified tissue fluoride. The radioactive fluoride concentration of the various skeletal tissues was approximately directly proportional to their proximity to the circulating blood. Urinary excretion and salivary secretion of the isotope occurred in appreciable amounts only when the blood concentration was elevated.

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THE NATURE AND LOCATION OF THE "SPHINCTER MECHANISM" IN THE LIVER AS DETERMINED BY DRUG ACTIONS AND VASCULAR INJECTIONS

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Ever since the early work of Stolnikow in 1882, the rôle of the liver as a reservoir for blood has been the subject of numerous investigations. Although it is generally conceded that the liver exerts a control over the blood flow through it, knowledge regarding the mechanism or mechanisms which contribute to the partial or total retention of blood in this reservoir is still fragmentary and conflicting.

The present investigation was undertaken in order to secure further information regarding the nature and location of the mechanism which controls the outflow of blood through the hepatic veins. Two methods of study were employed in this investigation: 1, perfusion of excised livers with various drug solutions (adrenalin, pilocarpin, physostigmin, atropine and acetyl-beta-methylcholine chloride); and 2, detailed microscopic examination of injected and cleared specimens obtained from livers previously perfused with the various drugs.

An important part of the discussion concerns two angioarchitectural features which seem to have entirely escaped the attention of earlier investigators. One of these features is the artery-like nature of the sublobular veins, and the other one is the structure which will be referred to as the "small sluice channel". The "small sluice channels" are peculiar side branches of the sublobular veins (figs. 7, 8, 9, 10, 11 and 12). They, together with the relatively thick walls of the sublobular veins, form a complex or multiple "sluice valve" which is probably the most important part of the mechanism which regulates the outflow of blood from the liver.

Material and technique. A comparative study was made of the livers of many laboratory and zoo mammals, but the results herein reported were obtained principally from surviving livers of adult cats and rabbits. The common laboratory animals were all killed by a sudden blow on the occiput. The others were killed by bleeding. In each case the liver was immediately exposed through a midline abdominal incision, the arteries to the intestines and stomach were ligated, and the lower end of the esoph-

agus was doubly tied and cut. The portal branches were ligated and a glass cannula was inserted into the portal vein as closely as possible to the hilus of the liver. After removing the stomach and intestines, the hepatic artery was ligatured and the abdominal vena cava was tied and then severed close to its entry into the liver. The thorax was opened and a second glass cannula was tied into the thoracic vena cava close to the diaphragm. The entire liver, with the diaphragm attached, was then removed and placed into an air plethysmograph which in all essential respects was like that described by Bauer, Dale, Poulsson and Richards (1932). The cannula in the portal vein was connected by means of rubber and glass tubing to a constant level reservoir containing warmed (39°C.) oxygenated dextrose-free Locke's solution. This reservoir was fixed at an appropriate level above the liver. All drug solutions were slowly administered from a burette or a series of burettes directly into the perfusion stream at a point close to the cannula which had been inserted into the portal vein. Solutions of varying H-ion concentrations were used in these experiments. It was observed that the most constant results were obtained when the pH of the perfusion fluid ranged between 7.8 and 8.15. An automatic tilting bucket was used to record the outflow of liquid from the liver.

At the completion of each perfusion experiment the liver was removed from the plethysmograph and the blood vessels in the organ were then injected with India ink alone, with India ink and vermilion cinnabar, or with vermilion cinnabar alone. Whenever a differential injection of the blood vessels was made, India ink was injected in a rhythmic manner and under low pressure (5 to 15 mm. of mercury) into the cannula which had been inserted into the inferior vena cava and then vermilion cinnabar (in water) was injected into the cannula inserted into the portal vein. All injected livers were cleared and otherwise prepared according to the methods described by Swindle (1935). The tissue was later sectioned and then examined with a binocular microscope. Some of the sections were mounted in dammar and photographed (figs. 7, 8, 9 and 10). Other sections were stained in order to determine some of the histological features of the blood vessels (figs. 11 and 12). All of the photomicrographs (figs. 7, 8, 9, 10, 11 and 12) were obtained by using the Eastman D-C Orthophotographic plate with transmitted light.

Results. *Effect of adrenalin.* Adrenalin chloride solution (P.D. & Co.) was used in quantities of 25 cc. of a 1 in 400,000 and a 1 in 60,000 solution. When the weaker solution of adrenalin was perfused by way of the portal vein through the liver of a rabbit, the outflow from the organ increased from 22.819 cc. per 100 grams per minute to 28.91 cc. per 100 grams per minute (fig. 1). This was an increase in outflow of 26.69 per cent. The total liver volume diminished 3.62 cc. Perfusion of the rabbit liver with

the 1 in 60,000 solution reduced the outflow from 26.77 cc. per 100 grams per minute to 10.12 cc. per 100 grams per minute, a decrease of 62.19 per cent (fig. 2). The total liver volume diminished 3.75 cc. Such contrasted

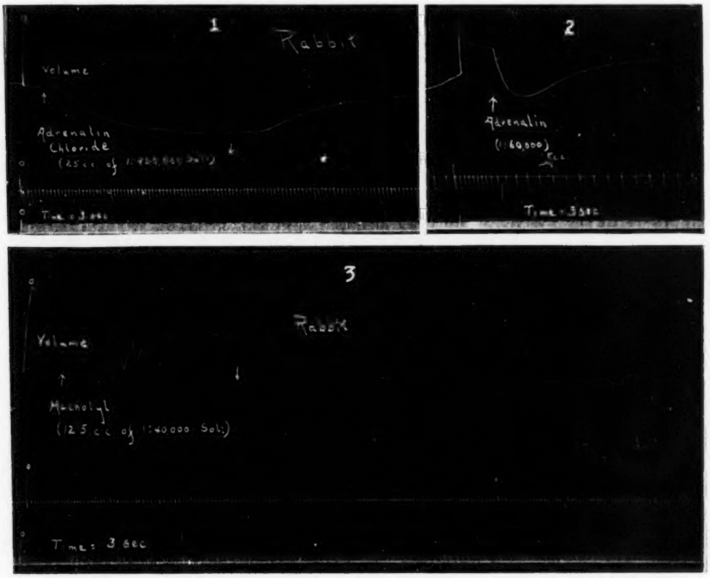


Fig. 1. Upper tracing, volume curve of the excised, surviving liver of an adult rabbit. Lower tracing, outflow of liquid through the inferior vena cava, each space between the perpendicular marks representing the outflow of 5 cc. of liquid. Perfusion of the liver by way of the portal vein with 25 cc. of a 1:400,000 adrenalin chloride solution reduced the liver volume 3.5 cc., and increased the outflow of liquid from the inferior vena cava 26.69 per cent.

Fig. 2. Same as figure 1 except that a 1:60,000 solution of adrenalin chloride was perfused through the liver. The liver volume was reduced 3.75 cc., and the outflow of liquid from the inferior vena cava was diminished 62.19 per cent.

Fig. 3. Upper tracing, volume curve of the excised, surviving liver of an adult rabbit. Lower tracing, outflow of liquid through the inferior vena cava, each space between the perpendicular marks representing the outflow of 4.5 cc. of liquid. The addition of 12.5 cc. of a 1:40,000 acetyl-beta-methylcholine chloride solution to the perfusion fluid increased the liver volume and diminished the outflow of liquid through the inferior vena cava.

effects of increased outflow after the smaller dose of adrenalin and decreased outflow after the larger dose of adrenalin were observed in experiments on nine cats, thirty-five rabbits and two vervet monkeys. It is of interest to note that the larger dose of adrenalin did not have a greater weakening effect on the resistance offered to the outflow than the smaller

dose, as shown by the almost identical diminution in liver volume in both instances.

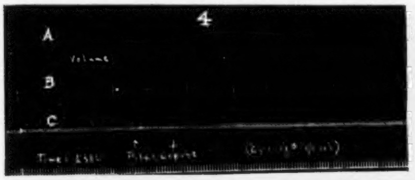
The injection of India ink by way of the inferior vena cava into livers which had been perfused with a small dose of adrenalin always produced very complete injections of the blood vessels in the organ, and the injection medium flowed freely out of the portal vein. In no instance was it necessary to use an injection pressure exceeding fifteen millimeters of mercury. When the blood vessels in any of the livers which had been perfused with a large dose of adrenalin were injected by way of the inferior vena cava, the liver expanded enormously and the outflow from the portal vein was observed to be very meager. Injection of the blood vessels of a liver by way of the portal vein, the organ having been previously perfused with a large dose of adrenalin, always required an injection pressure exceeding fifteen millimeters of mercury in order to force the injection medium into the liver lobules. This was not the case, however, when India ink was injected by way of the portal vein into livers which had been previously perfused with a small dose of adrenalin. In all instances India ink always flowed readily into the liver lobules even when the injection pressure was very low (5 mm. of mercury).

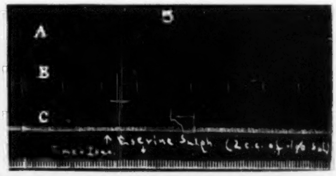
Effect of acetyl-beta-methylcholine chloride. Swelling of the liver and restriction of the outflow was regularly observed in rabbits (fig. 3), cats (fig. 6) and vervet monkeys when acetyl-beta-methylcholine chloride ("Mecholyl"-Merck) was perfused through the organ by way of the portal vein. As shown in figure 6, when 25 gamma of acetyl-beta-methylcholine chloride were perfused through a cat's liver, the liver volume increased 8.75 cc. and the hepatic outflow was reduced from 12.12 cc. per minute to 2.64 cc. per minute, a reduction of 78.2 per cent. The administration of 2 cc. of a 0.1 per cent solution of atropine sulphate while the liver was enormously expanded promptly checked and reduced the expansion of the organ, and the hepatic outflow became markedly accelerated.

The injection of India ink by way of the inferior vena cava into a liver which had been previously perfused with "Mecholyl" always produced very incomplete injections of the blood vessels in the organ (fig. 7). In many instances an injection pressure exceeding 150 mm. of mercury was found necessary in order to produce a free flow of the ink through the portal vein. Attempts to inject the liver by way of the portal vein always resulted in marked expansion of the organ and a meager flow of the injection medium from the inferior vena cava. When atropine sulphate had been perfused through the liver prior to the injection of the blood vessels in the organ, little or no resistance was offered to the flow of the ink. Only low injection pressures (5–10 mm. of mercury) were found necessary for thorough injection of these atropinized specimens.

Effect of physostigmin and pilocarpin. The results obtained by perfusing

cat and rabbit livers with either pilocarpin or physostigmin were in all essential respects similar to those obtained when acetyl-beta-methyl-





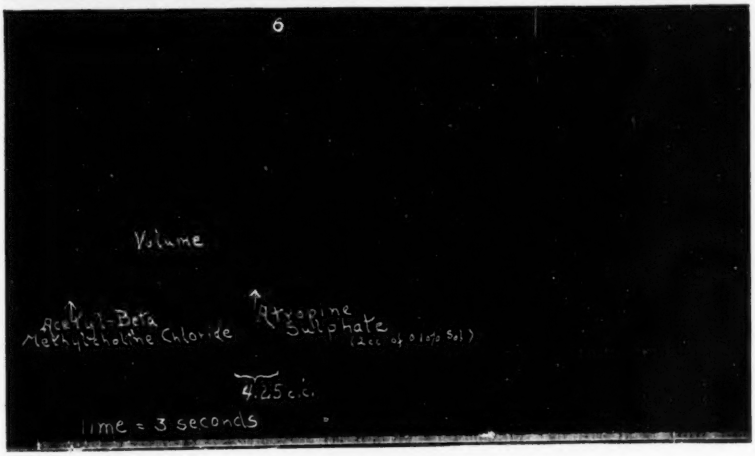


Fig. 4. Plethysmographic tracing obtained from the excised, surviving liver of an adult rabbit. The addition of 2 cc. of a 0.1 per cent pilocarpin hydrochloride solution caused an increase in liver volume and a decrease in liver outflow. A, volume curve of the liver; B, outflow of liquid from the inferior vena cava measured in 3.5 cc. volumes; C, the outflow in drops.

Fig. 5. Plethysmographic tracing from the excised, surviving liver of an adult rabbit showing the effect of perfusing 2 cc. of a 0.1 per cent eserine sulphate solution through the liver by way of the portal vein. The liver volume increased and the outflow from the inferior vena cava was reduced 29.98 per cent.

Fig. 6. Upper record, volume curve of the excised, surviving liver of an adult cat. Lower record, outflow of liquid through the inferior vena cava measured in 4.25 cc. volumes. When 25 gamma of acetyl-beta-methylcholine chloride solution were perfused through the liver by way of the portal vein, the volume of the liver increased 8.75 cc. and the outflow diminished 78.2 per cent. The addition of 2 cc. of a 0.1 per cent atropine sulphate solution checked and reduced the expansion of the organ, and the hepatic outflow became markedly accelerated.

choline chloride was added to the perfusing fluid. In all instances there was swelling of the liver and restriction of the outflow of liquid through the inferior vena cava. As shown in figure 5, when 2 cc. of a 0.1 per cent

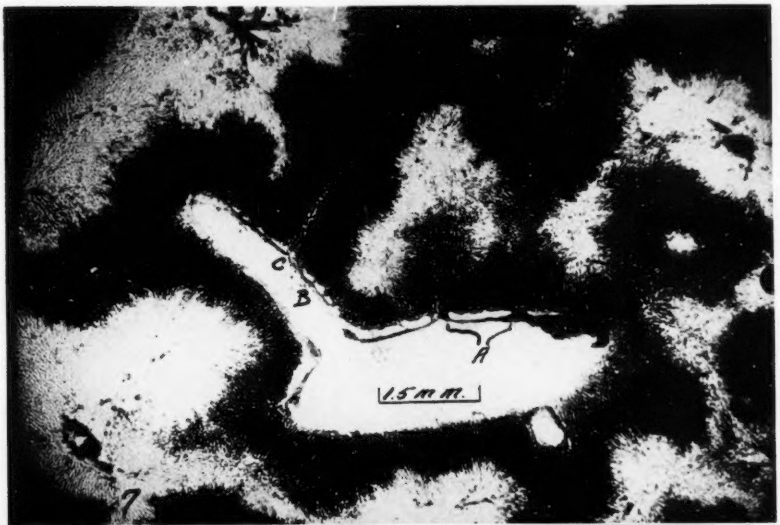
solution of physostigmin sulphate were administered by way of the portal vein, the flow from the rabbit liver was reduced from 14.74 cc. per 100 grams per minute to 10.32 cc. per 100 grams per minute, a decrease of 29.98 per cent. Similarly pilocarpin hydrochloride (fig. 4) reduced the outflow from the rabbit liver from 16.51 cc. per 100 grams per minute to 12.51 cc. per 100 grams per minute, a decrease of 24.22 per cent.

The results of injection of the blood vessels in livers perfused with either pilocarpin or eserine were similar to those obtained after the liver had been perfused with "Mecholyl." In no instance, however, was it necessary to exert a pressure greater than 40 mm. of mercury to obtain a free flow of liquid through the organ.

Microscopic examination of injected and cleared specimens. Detailed microscopic examination of the blood vessels in livers which have been perfused with either atropine or small doses of adrenalin reveals that the sublobular veins are markedly dilated and that they possess an unusually large number of peculiar side branches which are smaller but otherwise very similar morphologically to the central veins in the liver lobules. Each of these unusual side branches arises from the confluence of many ordinary sinusoidal capillaries. They penetrate more or less perpendicularly through all of the tunics of the sublobular vein (figs. 9 and 10). In this respect, these small side branches of the sublobular vein resemble the "small sluice channels" which were recently described in the erectile tissue of the penis (Deysach, 1939).

Relatively few or occasionally none of the unusual side branches can be observed piercing the sublobular veins in livers which have been perfused with acetyl-beta-methylcholine chloride, eserine or pilocarpin (figs. 7 and 8). The distribution of India ink in the liver lobules of these livers is such that only the sinusoidal capillaries in the immediate vicinity of the central veins are thoroughly filled. In contrast to this observation, however, whenever atropine or a small dose of adrenalin is perfused through the liver prior to the injection of the blood vessels, all of the sinusoidal capillaries in the liver lobules invariably become filled with the India ink (figs. 9 and 10).

Microscopic examination of injected and stained specimens obtained from the livers of cats, vervet monkeys and rabbits reveals that the hepatic veins and their branches are relatively thick walled and muscular (figs. 11 and 12), whereas the portal vein and its branches are very thin walled and poorly supplied with muscle tissue. The walls of the hepatic venous ramifications (e.g., the sublobular veins) are made up primarily of smooth muscle fibers, collagenous fibers, fibroblasts and a small amount of elastic fibers. The walls of the portal venous ramifications are made up of smooth muscle fibers, which are predominantly circularly arranged, elastic fibers, and a few collagenous fibers and fibroblasts. The smooth muscle fibers in the sublobular veins are arranged both longitudinally and circularly.



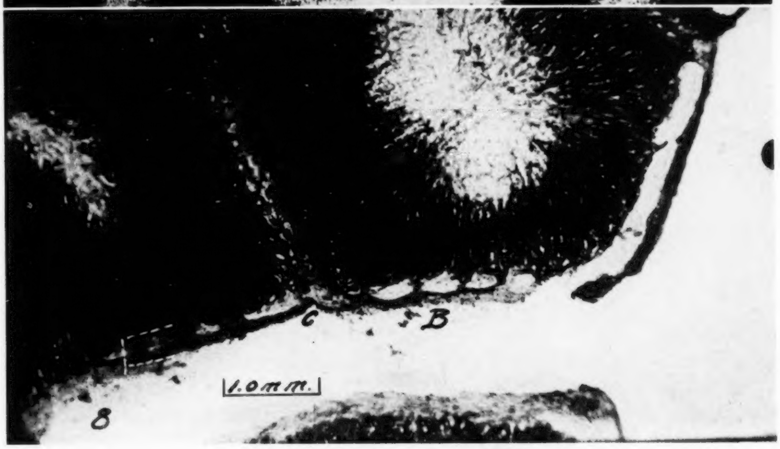
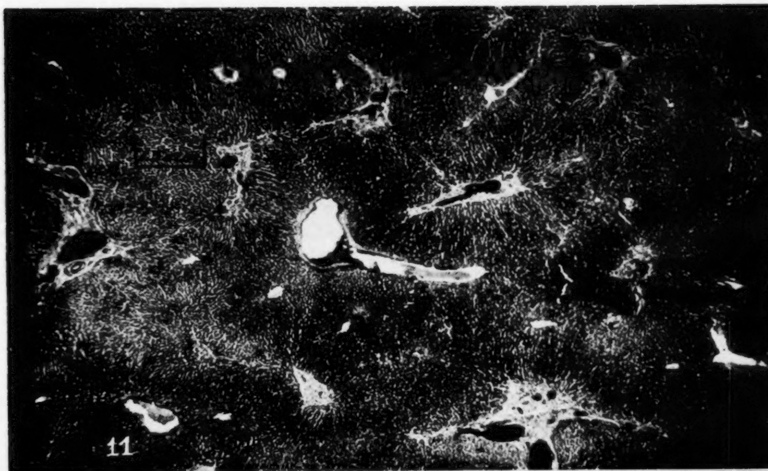


Fig. 7. Photomicrograph of a section obtained from an injected and cleared liver of an adult rabbit after the organ was perfused with 12.5 cc. of a 1:40,000 solution of acetyl-beta-methylcholine chloride. India ink was injected in a rhythmic manner and under low pressure into the inferior vena cava, and vermilion cinnabar (in water) was injected into the portal vein. A, small sluice channels closed; B, small sluice channels open; C, large, open sluide channels (central veins).

Fig. 8. Same as figure 7 except a relatively greater magnification was used in an effort to show the nature of the small sluice channels. The thickness of the wall of the sublobular vein is outlined by the broken lines made on the photographic print with pen and ink. It is interesting to note that the sinusoidal capillaries beyond the widely open sluice channels are thoroughly filled with ink.

Fig. 9. Photomicrograph of a section obtained from an injected and cleared liver of an adult cat after the organ was perfused with a 0.1 per cent atropine sulphate solution. The technique used in preparing this specimen was the same as for figure 7. Due to the fact that numerous small sluice channels were widely open, the sinusoidal capillaries in the liver lobules became thoroughly filled with the India ink solution.

Fig. 10. Same as figure 9 except a relatively great magnification was used in an effort to show the nature of the small sluice channels. All of the sublobular veins were markedly dilated throughout the specimen from which this section was obtained. Due to the relaxation of the musculature in the walls of the sublobular veins, India ink flowed freely through the widely open sluice channels and completely filled the sinusoidal capillaries in all of the liver lobules.



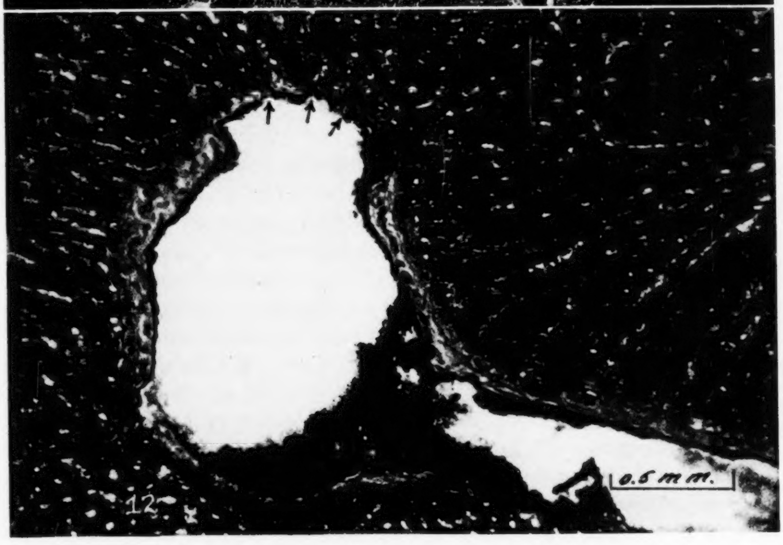


Fig. 11. Photomicrograph of an injected and stained specimen obtained from the liver of an adult rabbit after 12.5 cc. of a 1:40,000 solution of acetyl-beta-methylcholine chloride had been perfused through the organ. The technique used for injecting this specimen was the same as for figure 7. In the central part of the figure a central vein, cut longitudinally, is shown entering a sublobular vein, cut perpendicularly.

Fig. 12. Same as figure 11 except a relatively great magnification was used in an effort to show the microscopic structure of a sublobular vein. Three small sluice channels may be seen piercing more or less perpendicularly through all of the tunics of the sublobular vein. The small sluice channels arise from the confluence of numerous ordinary sinusoidal capillaries in the liver lobule and drain liquid directly into the lumen of the sublobular vein. The thick, muscular wall of the sublobular vein and the small sluice channels that pierce its walls form a complex or multiple "sluice valve."

The greatest number of longitudinally arranged muscle fibers form a layer next to the tunica interna. Externally to this layer some smooth muscle fibers are arranged both circularly and longitudinally. The circular muscle fibers are found predominantly between the internal longitudinally arranged fibers next to the tunica interna and a more external layer of longitudinally arranged fibers.

Peripherally to the walls of the sublobular vein, as shown in figure 12, the liver cells are arranged in a single layer or occasionally in a double layer. Numerous "small sluice channels" (single endothelial tubes) pierce between the cells of this systematically arranged layer around the sublobular vein and drain liquid from the liver lobule directly into the lumen of this vein.

Discussion. The anatomical evidence as to the nature of the mechanism responsible for regulating the outflow of blood from the liver has up to the present time been far less convincing than the physiological evidence of its existence. Bauer, Dale, Poulsson and Richards (1932) attribute the unusual behavior of the dog's liver under perfusion, and in response to chemical and nervous stimuli, to the presence of a strong muscular coat (sphincter) in the caval ends of the hepatic veins. Arey and Simonds (1920) state that the hepatic veins of the dog have "an enormous amount of smooth muscle in the wall, thus demonstrating an adequate anatomical basis for impeded vascular flow should spasm occur."

While the contraction of strategically placed non-striated muscle in the hepatic veins of the dog may serve as a satisfactory explanation for certain of the observed phenomena described by Simonds (1923), Popper (1931) and Bauer, Dale, Poulsson and Richards (1932), it does not elucidate the mechanism involved for the impounding of liquid in the liver of animals (cat, rabbit, vervet monkey, man, etc.) which do not possess unusually muscular hepatic veins.

In my own experiments the observations that physostigmin, pilocarpin and acetyl-beta-methylcholine chloride increase liver volume and decrease the outflow of liquid from the inferior vena cava strongly suggest the existence of a "resistance" (sluice valve) so located in the liver as to become effective after blood has passed through the sinusoidal capillaries. This point of view is also supported by the observations that atropine and small doses of adrenalin decrease liver volume and increase the outflow from the inferior vena cava. Corroborative evidence for the existence of such a "sluice valve" in the sublobular veins of the cat and rabbit, as well as in other animals thus far examined (raccoon, Virginia opossum, grizzly bear coati mundi, white-tailed deer and others), is found in my injected and cleared specimens (figs. 7, 8, 9, 10, 11 and 12).

Although it is generally stated that liquid enters the liver lobule by way of the "portal canals" and that liquid is drained from the liver lobule via

the central vein, my own observations show that liquid may also drain directly into the sublobular vein via small endothelial tubes (sluice channels) which arise from the confluence of many ordinary sinusoidal capillaries. As shown in some of my experiments, the outflow of liquid from the liver lobule is directly dependent upon the activity of the musculature in the walls of the sublobular veins. The size of the lumina of the small sluice channels varies greatly. This observation suggests that the small sluice channels probably open and close passively as the sublobular veins dilate and constrict. When the sublobular veins are markedly constricted, the flow of liquid through the small sluice channels is halted and the liquid is then shunted into the central veins of the liver lobules. It is both convenient and correct to speak of the central vein in each liver lobule as being a "large sluice channel". Although the lumens of the central veins also become greatly narrowed as a result of the constriction of the sublobular veins, in no instance during the course of this investigation have I ever observed their complete obliteration.

SUMMARY AND CONCLUSIONS

It is generally agreed that the "portal canals" are the gateways for blood entering the liver lobules. It is also generally agreed that the central veins drain liquid from the liver lobules into the sublobular veins. However, no mechanism has heretofore been described which adequately explains certain interesting variations of the flow of blood through the liver. Evidence for the existence of an adequate mechanism (the sluice valve) was presented in the present paper. This evidence was secured by observing the effects of certain drugs and also by examining microscopically various liver specimens after they were perfused with various drug solutions, later injected with India ink or vermilion cinnabar (in water), and then cleared. Stained preparations from the specimens were also examined.

When small dosages of adrenalin are perfused by way of the portal vein through the excised, surviving liver of the rabbit, cat,and vervet monkey, the outflow of liquid through the inferior vena cava becomes markedly accelerated and the volume of the organ diminishes. Atropine produces a similar result. Large doses of adrenalin reduce hepatic outflow and diminish liver volume. On the other hand, pilocarpin, eserine and acetyl-beta-methylcholine chloride ("Mecholyl"-Merck) produce swelling of the liver and reduction of the outflow of liquid through the inferior vena cava.

The effects of pilocarpin, eserine, acetyl-beta-methylcholine chloride and small doses of adrenalin can be explained in terms of the action of a complex valve consisting of the walls of the sublobular veins and the small and large endothelial tubes (sluice channels) which enter these veins more or less perpendicularly. Pilocarpin, eserine and acetyl-beta-methylcholine chloride (in proper dosage) cause the musculature in the walls of the sub-

lobular veins to contract and thereby close (partially or totally) those portions of the endothelial tubes which are situated in the walls of these veins. The lumens of the large endothelial tubes (the central veins) also become greatly narrowed as the result of the contraction of the musculature in the walls of the sublobular veins. The "sluice valve" may be opened up widely by atropine or small doses of adrenalin. In no instance was it possible to observe anything resembling a valvular mechanism in either the walls of the portal venous radicles or in the immediate vicinity of these radicles.

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STUDIES ON TURTLE HEARTS—THE END OF SYSTOLE, THE DURATION OF THE REFRACTORY PERIOD, THE LATENT PERIOD OF EXTRASYSTOLES AND THE INFLUENCE OF HEART RATE ON AORTIC BLOOD PRESSURE

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On a summer day turtles, lying on a log in the sunshine, may have body temperatures of 30 to 35°C. In that range their metabolic and heart rates are probably elevated to values fifteen to twenty times those present at 0 to 3°C. (Clark, 1927). Accompanying the elevated metabolism, oxygen transport to tissues may be augmented by utilizing a larger amount of arterial oxygen and by increasing the blood flow. In turtles, how important is this latter mechanism and to what extent is it produced by elevation of the heart rate?

Recently, Shannon and Wiggers (1940) published aortic and ventricular pressure pulses and electrocardiograms from turtles whose hearts were artificially accelerated by "break" shocks. Nothing was done to modify the peripheral resistance or the venous return to the heart. They reported: 1st, under conditions assuring normal effective venous pressure the turtle and frog ventricle, unlike those of mammals, does not increase the minute output by an increase in rate; 2nd, the optimal heart rate is 30 to 40 beats per minute; 3rd, any further increase in rate reduces the systolic and diastolic blood pressure from which they concluded that the minute cardiac output is reduced; and 4th, the turtle ventricle is not refractory during the last third of systole.

Woodbury and Hamilton (1937) published carotid and ventricular pressure pulses of turtles with slightly higher pressures than those of Shannon and Wiggers (1940). In view of the fact that these higher pressures were obtained from turtles whose hearts were beating at nearly twice the reported "optimal rate", studies on the turtle have been continued.

Optical records of the blood pressure of nine unpithed turtles were obtained with the Hamilton "hypodermic" manometer in the manner previously described (Woodbury and Hamilton, 1937).

Acceleration of the Heart by Rythmic Electrical Stimuli. This produced

pressure changes similar to those reported by Shannon and Wiggers (1940). Small increases in the rate narrowed the aortic pulse pressure and produced small if any change in the mean pressure. Marked acceleration of the heart not only narrowed the pulse pressure but lowered the mean pressure. In some cases the diastolic as well as the systolic pressure was reduced.

These results, however, do not supply data concerning the effect of increased heart rate *by itself* upon cardiac output. When the heart is accelerated by electrical stimuli, systole is not shortened. This abnormally shortens diastole and the filling time of the ventricle. In turtles this increase in the total systolic time per minute also diverts blood into the pulmonary artery (unpublished data). Therefore, these changes in aortic blood pressure fail to supply any data concerning ventricular output.

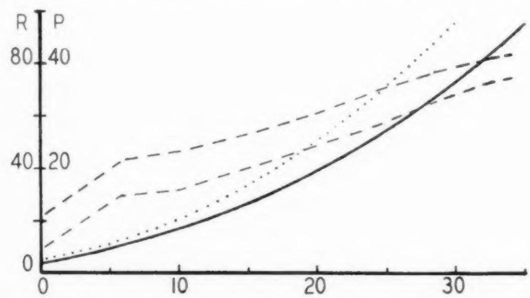


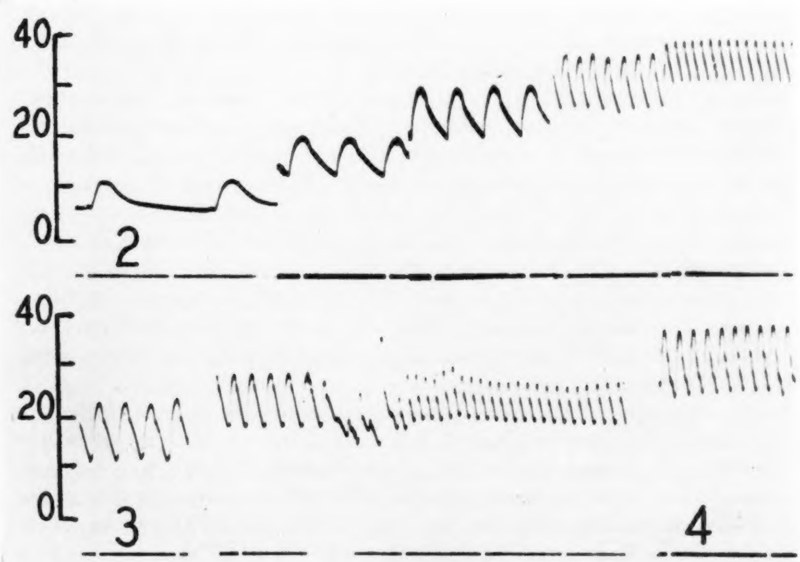
Fig. 1. Plot showing the relationship in turtles (average of six animals) between the body temperature and the heart rate (solid line) and systolic and diastolic blood pressure (broken line). The dotted line reproduces the results of Clark (1920) on the isolated frog heart. Ordinates, P = blood pressure in millimeters Hg; and R = heart rate per minute. Abscissae, body temperature.

These results also fail to determine the "optimal heart rate" under physiological conditions. Accelerating the heart artificially by means of electrical stimuli in addition to its above effects did not produce physiological changes in the vascular system or in the circulatory demands of the body. The heart, under normal regulation and coördinated to perform its normal work, may have a very different "optimal rate" from one accelerated out of all relation to the circulatory demands of the organism.

Acceleration of the Heart by Increasing the Temperature. The hearts of these same turtles were accelerated in a natural manner. This was accomplished by warming the entire turtle with water baths at temperatures up to 35°C. As shown in figure 1, the aortic blood pressure values progressively increased as the heart was accelerated. At similar heart rates the pressure values correspond very well with those previously published (Woodbury and Hamilton, 1937; Shannon and Wiggers, 1940).

Like mammals and birds (Clark, 1927), the amphibia and reptiles apparently have slower heart rates in the larger species.

At a given pressure, the rate of diastolic descent is somewhat greater at higher temperatures and at more rapid heart rates (compare the last three records of fig. 2). This is good evidence of vasodilatation and increased rate of outflow through the arterioles, if the remote possibility



Figs. 2–4. Pressure pulses from innominate artery. All records are from the same turtle. Blood pressure scales show millimeters Hg. Time intervals are 10 seconds.

Fig. 2. Records taken at body temperatures of approximately 0°, 8°, 10°, 18° and 28°C.

Fig. 3. The water bath was kept constant at 11°C. At break in record (elapse of 30 sec.) the heart was warmed with Ringer's solution at temperature of 30°C. At signal on base line, 38°C. Ringer's was poured upon the heart.

Fig. 4. Ten minutes later when the heart and body of turtle were nearly the same temperature (28°C.).

of constriction of the large arteries ("Windkessel") is disregarded. In spite of this vasodilatation the blood pressure did not decrease, but definitely increased (see figs. 1 and 2). Only an increase in the cardiac output into the aorta could account for the rise in blood pressure. The "optimal heart rate" for pumping blood into the aorta was not reached even at 100 beats per minute.

Warming the entire turtle affects the vascular system by warming the

body tissues and by warming the heart. Warming the body tissues increased tissue metabolism (Clark, 1927), peripheral blood flow and systemic venous return to the heart. Warming the heart increased cardiac metabolism (Clark, 1927), the speed of muscular contraction, the rate of systolic outflow and the heart rate without allowing systole to encroach abnormally upon diastole (see fig. 3).

When the heart rate is accelerated by warming the entire animal, these changes provide increased venous return, adequate time for diastolic filling, increased minute cardiac output and a rise in the aortic blood pressure values (see figs. 1 and 2).

Keeping the body temperature constant and warming only the heart should give data concerning the effect of increasing the heart rate *by itself*. This method increased the heart rate without changing the rate of diastolic descent at any given pressure (see fig. 3). This means that peripheral vasodilatation had not occurred and was not increasing the systemic venous flow. The increased speed and rate of cardiac contraction would be expected to increase the systolic and diastolic pressures until the systemic and pulmonary reservoirs are depleted. As shown in figure 3, this happened. Further increases in the rate and speed of contraction narrowed the pulse pressure, reduced systolic pressure and produced a very slight rise in the diastolic pressure.

Since there is no definite fall in mean pressure, the heart rate was not increased beyond the "optimum". Even at the rate of 48 beats per minute the circulation was maintained at a level which is certainly as great as at slower rates. The narrowed pulse pressure indicates a venous return that is not commensurate with the increased heart rate. The venous return limits the cardiac output, because when the whole body of this turtle is warmed up to a temperature which corresponds to that of the heart, the blood pressure goes up, the pulse pressure widens and circulation is increased (fig. 4). Further acceleration by warming only the heart again narrowed the pulse pressure and produced changes similar to those in figure 3. Raising or lowering the body temperature of the animal raised or lowered the heart rate value beyond which further artificial increases in the heart rate failed to increase the blood pressure.

The "optimal rate" of the turtle heart is at the arbitrary choice of the experimenter when he makes the conditions of the experiments.

THE END OF SYSTOLE, THE REFRACTORY PERIOD AND THE CHARACTERISTICS OF EXTRASYSTOLES. According to Woodsworth (1903), Marey from his classical experiments concluded that the refractory period shrank as the stimulus was strengthened, being gradually reduced to the first instants of systole and finally disappearing. Hildebrand (1877), Engelman (1895) and Woodsworth (1903) presented the definite proof that the delayed contractions caused by strong stimuli during systole were secondary

to auricular contractions arising from a spread of current. It is these latter studies that should be credited as providing the basis of our present conception that the absolutely refractory period continues to the end of systole.

Simultaneous auricular and ventricular myograms from six turtles and simultaneous pressure pulses and electrocardiograms from nine turtles supplied most of the data for this portion of the paper. Approximately two thousand strong electrical stimuli from an inductorium were applied at various times during the cardiac cycle.

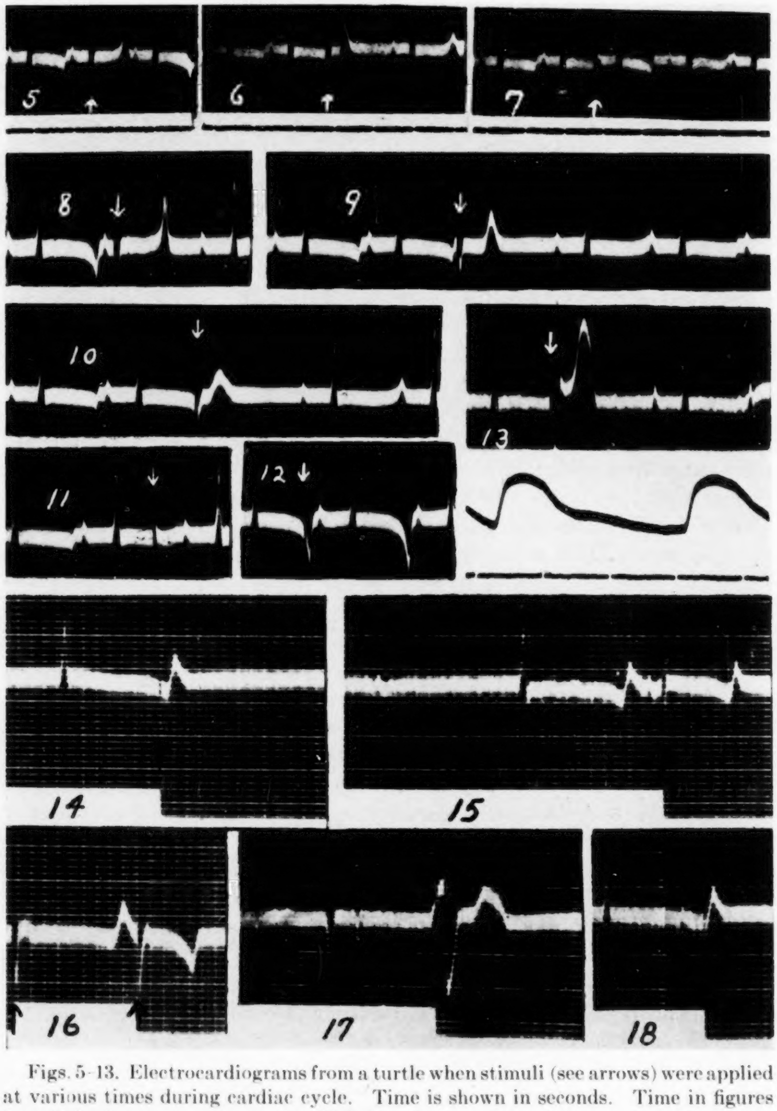
Actual Time of Stimulation. At each break of the primary circuit, two stimuli really are sent to the ventricle. Either the *beginning* or the *cessation* of current flow in the secondary circuit can serve as the stimulus. Generally the beginning of flow is the effective stimulus and the cessation of flow occurs during the refractory period of the response. However, as the "break" shocks are applied earlier in the cardiac cycle, there is a period of time when the beginning of flow would be in the last portion of the refractory period of the regular contraction. The cessation of flow could then occur after the end of the refractory period and could excite the ventricle. For this reason the author has used the time of *cessation* of flow in the secondary circuit instead of the time of the "break" of the primary circuit for obtaining data concerning the refractory period.

Electrocardiograms clearly demonstrate that these two stimuli are separated by a definite but small period of time (see figs. 7, 12, 14 and 18). When using nonpolarizing electrodes and an inductorium, the time interval was 0.03 to 0.04 second. This time interval was much longer and occupied from one-fifth to one-sixth of systole in the electrocardiograms published by Shannon and Wiggers (1940). Evidently they used galvanic current. Yet, for their data concerning the refractory period, they used the beginning of current flow as the time of stimulation and disregarded the second stimulus (the cessation of current flow).

The End of Systole. Myograms and pressure pulses record the duration of systole of the ventricle as a unit or pump. On the other hand, the T wave of the electrocardiogram provides a better criterion for the ending of systole within the ventricular tissue.

At the start of an experiment, the first portion of the T wave generally corresponded fairly well with the end of mechanical systole (fig. 19). Experimental procedures, however, disturbed this relationship.

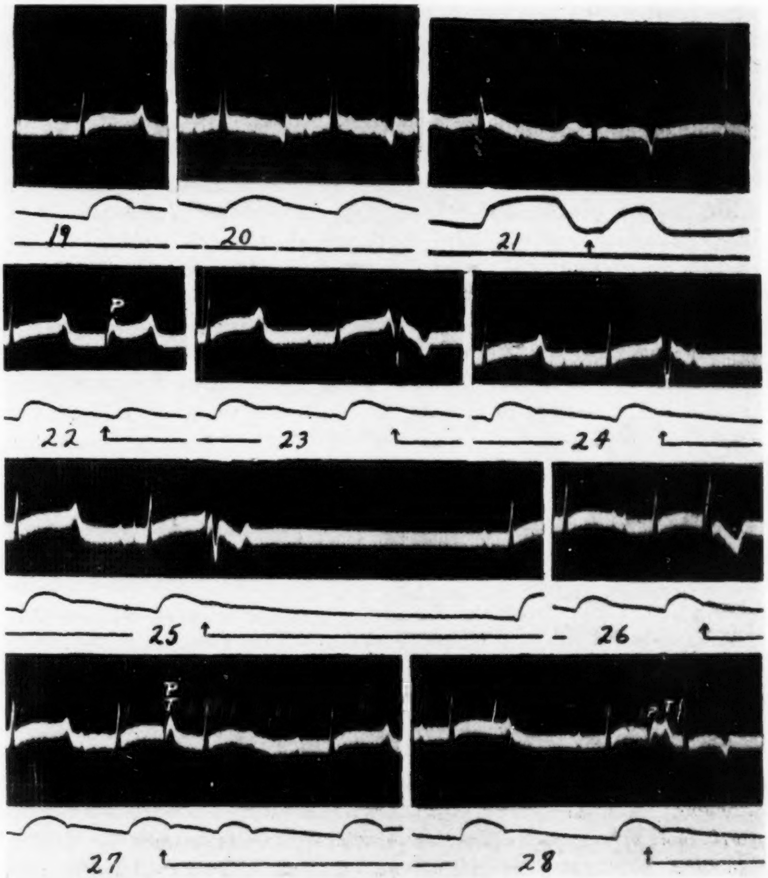
Frequently after applying a strong stimulus, the T waves of the next few regular cycles became abnormal. They started earlier in the cycle and were either heightened, flattened or inverted (figs. 5, 6, 7, 9 and 10). This early appearance and the resulting lengthening of the T wave soon persisted in most of the experiments (figs. 20, 21 and 27). This is interpreted by the author to indicate that systole was now ending prematurely



Figs. 5–13. Electrocardiograms from a turtle when stimuli (see arrows) were applied at various times during cardiac cycle. Time is shown in seconds. Time in figures 8 to 13 is the same as figure 13. The stimulating electrodes were large but were close together.

Figs. 14–18. Electrocardiogram from a turtle with complete heart block. The stimulating electrodes were large and widely separated. The arrows indicate time of stimulation.

in some small portions of the ventricle. Even though mechanical systole was still present, diastole was starting in this small portion of the heart.



Figs. 19-21. Simultaneous electrocardiograms and aortic pressure pulses. Those in 19 and 20 were taken early in experiment, those in 21 were obtained after complete heart block.

Figs. 22-28. Simultaneous electrocardiograms and aortic pressure pulses from turtle supplying records in figure 19. Time = 0.2 sec. Stimulating electrodes were large and widely separated. The arrows indicate the time of stimulation. *P* = P wave of extrasystole produced by current spread. *T* = regular T wave.

When comparing the duration of the refractory period with that of systole, the time when systole first ceases in any portion of the ventricle

should be considered as the end of systole. Therefore, the beginning of the T wave has been used by the author as the criterion for the end of systole.

The Form of the Ventricular Complexes. Influence of location of the stimulating electrodes. When the stimulating electrodes were close together two waves were generally present (see figs. 5, 6, 8, 9, 10 and 13). The spread of the wave of negativity gave rise to a modified Q R S wave. The disappearance of the negativity produced a T wave. Of course, the actual form of these waves varied with the location of the stimulating electrodes. When the electrodes were large and widely separated, stimulation in mid or late diastole produced a different type of electrocardiogram. The T wave was present, but the Q R S wave was absent or very short in duration (figs. 15 and 22). With large electrodes placed far apart the strong stimuli produced sufficient spread of current to excite simultaneously the entire ventricle. Of course no Q R S deflection occurred for the heart was still iso-electric even though it was responding to the excitation current. As the extrasystole ended and as the negativity of the heart disappeared, the T wave was produced. The fact that there was a T wave is significant. It proves that under the conditions of the experiment the duration of systole varies in different parts of the ventricle of turtles as in dogs (Wilson and Herrmann, 1921).

Effect of applying the stimuli earlier in diastole. As in dogs (Wiggers, 1925) the extrasystoles became shorter (see figs. 5, 6, 8 and 9). The T wave became more prolonged. The electrical latent period did not increase which differs from the accepted observations (discussed below).

Additional and more pronounced changes were present, if the heart had been subjected to many strong electrical stimulations and if the T waves of the regular heart beats developed during mechanical systole. Excitation early in diastole (during the early part of the abnormal regular T wave) produced extrasystoles with prolonged and often bizarre Q R S and T waves. These prolonged Q R S waves varied in form between two extremes. At times (figs. 13, 16, 23 and 24) the wave of negativity merely spread very slowly over the ventricle and simply prolonged the Q R S wave. At other times (figs. 17, 25 and 26) the early part of the wave was small and there was a definite delay before the wave spread over the rest of the ventricle. This produced a bizarre, often diphasic, Q R S wave. Both types of Q R S waves indicate that only certain portions of the ventricle responded to the excitation current. As adjoining portions finished systole and became capable of excitation, the wave of negativity spread until the whole ventricle was stimulated.

Associated with these unusual electrocardiograms, fused contractions like those reported by Cushny and Matthews (1897), delayed contractions and reciprocal ventricular contractions were recorded. In animals with

larger hearts, are such abnormal prolongations of the T waves in the regular contractions associated with the appearance of the vulnerable period? In dogs, Wegria and Wiggers (1940) report that there is a vulnerable period during the last portion of mechanical systole (0.03–0.06 sec.) when strong stimuli will produce ventricular fibrillation. According to their published records this appears to correspond to the period of time during the development of the T wave.

The Latent Period of the Ventricular Extrasystoles. Contrary to the accepted belief, the latent period of cardiac muscle does not significantly increase as the stimuli are applied earlier in the non-refractory period. Throughout most of diastole, stimuli produced electrical responses which

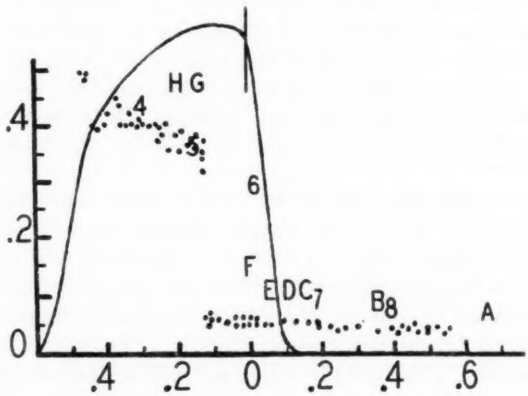


Fig. 29. Plots. Ordinates, duration of the latent periods in seconds. A ventricular myogram has been sketched on the plots. Dots = data from author's records, the A-V conduction time in this animal was 0.3 sec.; letters = data from corresponding records in figure 4 of Shannon and Wiggers (1940); and numbers = data from Marey's classical myograms.

quickly spread over the ventricle (figs. 5, 6, 8, 9 and 10). Myograms and pressure pulses as well as the electrocardiograms showed very short latent periods (fig. 29). In very early diastole, stimuli also produced an *immediate* response which frequently can be demonstrated only on the electrocardiogram. A comparison of figures 7 and 12 with 13, figures 14 and 18 with 17 and figure 27 with 24 to 26 shows that if a ventricular response occurred, the Q R S wave started immediately. The first portion may be small, but it was present and modified the regular T wave. This proves that the electrical latent period of cardiac tissue is not significantly increased as the stimuli are applied earlier in the cycle.

Since the spread of the extrasystole impulse was slow, delayed and abnormal, myograms and pressure pulses (figs. 13 and 29) showed the accepted

lengthening of the latent period. However, this increase in the latent period of the heart as a pump, was very much less than the amount stated by Marey (Woodsworth, 1903) and Shannon and Wiggers (1940).

Reproductions of Marey's classical myograms still appear in several recent textbooks of physiology. These are said to illustrate the duration of the refractory period in the heart and the gradual increase in the latent period as the stimuli are applied earlier in the cycle (Howell, 1940; Macleod, 1938; and Starling, 1936). They actually illustrate neither of these. Assuming a heart rate of 40, time on these records has been estimated and the lengths of the latent periods have been plotted (see fig. 29). Contractions 4 and 5 appear to be the result of auricular contractions produced by a spread of current (Woodsworth, 1902–03; and Best and Taylor, 1939). The delay associated with the A-V conduction would account for their long latent period. Contraction 6 resembles those with prolonged Q R S waves where the first portion of the wave is small and the main deflection of the Q R S wave is delayed (see figs. 17, 25 and 26). In the remaining extrasystoles (7 and 8) the latent periods show no significant change in duration.

Ventricular extrasystoles originating by A-V conduction produced aortic pressure pulses (figs. 27 and 28) almost identical to some published by Shannon and Wiggers (1940). Careful measurements were made of the latent periods of these extrasystoles. These data are plotted and presented in figure 29 and fail to convince the author that their extrasystoles *G* and *H* were ventricular in origin.

The presence of a compensatory pause after extrasystoles does not serve as circumstantial proof that current spread to the auricle did not occur. The presence of this pause only proves that the current did not spread to the pacemaker, the sinus venosus in turtles. As shown in figures 7 and 27, the compensatory pause is present even though the electrocardiogram shows that current spread to the auricle does occur.

The Refractory Period. As in dogs (Wiggers, 1925) the turtle ventricle is capable of excitation during the P-R interval (see figs. 5 and 8).

Many records similar to those in figures 5 to 28 provide clear demonstrations of the continuation of the refractory period until and including the very earliest portions of the T wave. The application of the excitation current just prior to the beginning of the T wave and while the entire ventricle was still in systole (figs. 7 and 27) never produced a direct stimulation of the ventricle even though at times the excitation current originated from full strength "break" shock from an inductorium with 10 batteries in series in the primary circuit. A large proportion of the stimuli applied slightly later but definitely during the first portion of the T wave produced direct ventricular contractions (see above). However, at this time diastole has begun in some portions of the ventricle, even though the mechanical systole has not been completed.

No evidence has been observed that ventricular tissue was excitable before the end of electrical systole in some portions of the heart.

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CONCLUSIONS

1. In turtles when the heart rate is modified in an artificial manner (local heat and electrical stimuli) there is lack of coördination between cardiac pumping and the vascular system.

2. When the heart is accelerated artificially, the so-called "optimal heart rate" can be raised or lowered by raising or lowering the body temperature.

3. When the heart rate increases are governed by body temperature and are coördinated with increased venous return and metabolic needs, the "optimal heart rate" is well above 70 beats per minute.

4. In the intact turtle, increasing the heart rate from 2 to 70 beats per minute by means of a natural stimulus (elevating the body temperature from 0 to 30°C.) markedly increased the systolic and diastolic pressure. This occurred in spite of vasodilatation and indicates a progressive increase in cardiac output.

5. Intact turtles possess a very effective mechanism for increasing blood flow in the systemic circulation. This is accomplished by vasodilatation, an increase in cardiac output and elevation of the blood pressure. The increased rate and speed of cardiac contraction are two important factors contributing to the increased minute cardiac output into the systemic circulation.

6. The relationship between the T wave and the end of mechanical systole differs from that generally recognized. Usually the T wave started just before the end of mechanical systole. Experimental procedures disturbed this relationship. Then the T wave would start somewhat earlier in the cycle and would be prolonged.

7. Under experimental conditions the duration of systole differs in different portions of the turtle ventricle. Prolonged repeated experimental procedures generally increased this difference.

8. Ventricular tissue is absolutely refractory to stimuli until the end of electrical systole in some portion of the ventricle.

9. As the excitation current was applied earlier in diastole, the following changes occurred.

a. Myograms and pressure pulses showed an increase in the latent period of the extrasystoles.

b. Electrocardiograms showed that the latent period of the ventricle tissue did not increase. This is contrary to the present accepted understanding.

c. The Q R S and T waves were prolonged.

d. The duration of the extrasystole decreased as previously reported.

10. Stimulation of the ventricle during the early portions of the T wave produced either fused ventricular contractions, delayed ventricular contractions or reciprocal ventricular contraction. An explanation of their production is given. In animals with larger hearts, similar conditions very likely produce ventricular fibrillation.

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RECOVERY OF FUNCTION FOLLOWING ARREST OF THE BRAIN CIRCULATION[1, 2]

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A critical analysis of the various methods which have been employed to investigate the effects of experimental arrest of the brain circulation reveals that most of these methods are incapable of yielding accurate information (1, 2). Recently, Weinberger, Gibbon and Gibbon (1) stopped the entire circulation by clamping the pulmonary artery in cats. This method has produced quite consistent results, but it has the disadvantage of depressing the function of other vital organs such as the heart, the lungs and the kidneys.

A method has already been described which brings about cessation of cephalic blood flow in the dog (3, 4), utilizing the principle of a cervical pressure cuff. The arrest of cephalic circulation is complete and the remainder of the body is maintained in good condition by adequate oxygen supply and circulation throughout the procedure.

The results have been consistent from dog to dog when the same period of brain stasis was employed. The uniformity of the effects of this procedure in normal adult dogs of both sexes has formed the basis of the demonstration of the greater resistance of young animals (5) and the decreased resistance of pregnant or lactating females (6) to arrest of the brain circulation.

RESULTS. *A. Disappearance of responses during acute arrest of the brain circulation.* Complete arrest of the brain circulation was produced in 31 adult dogs for periods ranging from 2–11$\frac{1}{4}$ minutes. The corneal reflex as a rule disappeared in 10 to 20 seconds but in several experiments could no longer be elicited 5 seconds after cervical compression. Respiratory movements ceased 15 to 20 seconds after compression in most instances and never persisted longer than 30 seconds. Urination frequently

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occurred during the first minute. The pulse was strong and rapid during the first few minutes but became weaker as the stasis was continued. After $1\frac{1}{2}$ to 2 minutes of stasis, the animal relaxed, no spontaneous movements of any type were observed and profound spinal shock became evident.

B. Behavior of surviving animals. The results were constant from dog to dog and there was no evidence that the effects were influenced by the sex, weight, breed or age of the normal adult animal. All animals which survived a period of arrest of brain circulation of 6 minutes or less recovered function apparently completely, while animals which were subjected to periods of stasis of 8 minutes or longer invariably suffered permanent damage to the brain.

The course of recovery following various periods of brain stasis is essentially similar except for differences in time relations dependent on the duration of the stasis. The course of recovery can be divided conveniently into the following periods: 1, period of early return of function; 2, period of hyperactive coma; 3, period of quiescent coma; 4, period of apathy and severe ataxia; 5, period of residual ataxia; 6, recovery. All of the experimental animals went through the first three periods but only animals surviving 6 minutes or less of brain stasis showed the last three periods of recovery.

1. Period of early return of function. Throughout this period, the dog remained in coma and was flaccid. The average figures for return of function of respiration and the wink reflex are illustrated in figure 1. The recovery time of the corneal reflex was four times that of the respiratory center. Recovery time increased sharply when the arrest of circulation exceeded the critical period of 7 minutes.

The threshold of spinal reflexes was initially high and fell progressively, indicating a gradually receding spinal shock. At first, stimulation was effective in eliciting only the homolateral flexion reflex and only some time later was it possible to produce a crossed extension reflex. In one experiment, after an 8-minute period of brain stasis, the crossed extension reflex first appeared 50 minutes after restoration of blood flow in the brain.

Shivering was a frequent occurrence during the early hours after the cephalic stasis, even though the rectal temperature was normal or elevated. The rectal temperature was, as a rule, only slightly above normal in the first hours after restoration of blood flow, rising to 102 to 103°F., whereas the normal rectal temperature ranged from 101 to 102°F.

In one dog, the circulation to the brain was stopped for 11 minutes and 15 seconds and the animal survived for only 2 hours. This experiment is of interest because the rectal temperature in this animal rose to 110°F., indicating acute failure of the heat-regulating mechanisms. The animal showed gasping respiration during this period and the corneal reflex

never returned. The pupils gradually constricted and at 25 minutes after restoration of blood flow, became 1 mm. in diameter and remained so until death. The dog regained the flexion reflex but only a weak crossed-extension reflex.

2. Period of hyperactive coma. A characteristic phenomenon which appeared some time after resuscitation and persisted for several hours was the occurrence of rapid running movements of all limbs, often accompanied by salivation and vocalization. These movements, which were well-

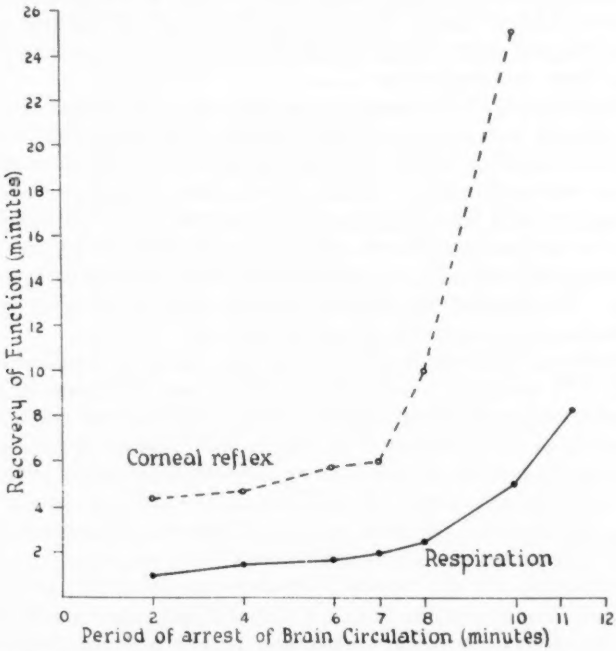

Fig. 1. Recovery of function of the respiratory center and of the corneal reflex following arrest of the brain circulation in adult dogs. The points represent average values.

coördinated and rhythmic, were carried out with the dog lying unconscious on his side. The movements were occasionally interrupted spontaneously. During a quiescent period, tactile or visual stimuli caused a resumption of the running movements. There was hyperirritability of knee jerks.

Early in the period of hyperactive coma, some extensor rigidity was observed. The head usually showed opisthotonus and the jaws were closed rigidly. In intervals between running movements, moderate extensor rigidity was evident in the limbs, predominantly in the forelimbs.

Even in cases in which the extensor rigidity was rather marked, however, it was insufficient to support the weight of the body. Labyrinthine righting reflexes were absent. Auditory stimuli were ineffective and visual reflexes included constriction of the pupils and lid movements.

No convulsions were observed following *complete* arrest of the brain circulation. On the other hand, in one case in which the blood flow through the brain was stopped *incompletely* for 15 minutes, gradually increasing convulsions were seen, culminating in status epilepticus.[3]

3. Period of quiescent coma. Coma persisted for a long time even after brief arrest of blood flow in the brain (table 1): after 2 minutes of stasis for 12 to 18 hours; after 6 minutes, for 24 hours or longer; after 8 or more minutes, coma was permanent.

The characteristics of quiescent coma were observed in all of the experimental animals, but were most easily studied in the dogs subjected to the longer periods of brain stasis. The animal showed no spontaneous movements and was unconscious. Auditory and olfactory reflexes were absent while pupillary and lid reflexes to light were present. The threshold of response to noxious stimulation was greatly elevated, and there was no localization of the stimulus, no emotional reaction or integrated purposive response. The homolateral rhythmic scratch reflex of the hind leg could be elicited but the movement was poorly directed.

The limbs and tail were flexed, the neck was rigid, the head moderately extended with masseter muscle spasm. There was resistance to passive extension of the flexed limbs and tail. When the limbs or tail were passively extended, they returned slowly to the original flexed position. Rigidity was present in the proximal but absent in the distal joints of all limbs. With the dog lying on his side, the flexor rigidity was equal on the two sides of the body. When the animal was supported in space in various positions or the head was rotated, he maintained a constant posture, but typical catatonia did not appear. Pressure on the foot pad failed to evoke reflex extension of the limb. Vestibular and righting reflexes were absent. In some experiments with longer periods of brain stasis, a persistent spontaneous rapid vertical nystagmus was evident for several days. The knee jerks were hyperactive.

Despite the persistent coma, complex reactions associated with feeding and elimination, which were absent during earlier periods of recovery, were regained during this period. When the head was supported and the mouth held in milk, the animal lapped mechanically and could be fed in this way.

[3] In this dog, periodic spasmodic twitching of the hind limbs and mouth began 27 minutes after restoration of blood flow. The convulsions developed progressively until 1½ hours after the stasis when the dog showed violent spasms occurring in very rapid succession. The convulsions were clonic in character, with simultaneous spasms of the limbs, tail, ears and mouth. Even the pupils participated in the convulsions with a synchronous marked hippus.

TABLE 1

Effects of various periods of arrest of the brain circulation in normal adult dogs

DOG	SEX	PERIOD OF BRAIN STASIS	SURVIVAL	DURATION OF COMA	POSTURE	PERSISTENCE OF APATHY	PERSISTENCE OF ATAXIA	END RESULT AND COMMENT
		minutes		*hours*		*days*	*days*	
1	M	2	4 months	12-18	Can stand after 18 hours	2	7	Apparently normal
2	F	2	4 months	12-18	Can stand after 18 hours	2	5	Apparently normal
3	F	4	19 days	18	Can stand after 18 hours	4	19	Apparently normal except for very slight ataxia
4	M	4	3 months	18	Can sit up on 2nd day. Can stand on 3rd day	4	21	Apparently normal
5	M	4	4 months	24	Can sit up after 24 hours. Can stand after 36 hours	3	18	Apparently normal
6	F	6	Still alive (1 year)	40	Can sit up after 24 hours. Can stand after 40 hours	4	40	Apparently normal
7	M	6	3 months	24	Can sit up after 24 hours. Can stand after 48 hours	3	20	Apparently normal
8	F	6	3 months	24	Can sit up after 24 hours. Can stand after 48 hours	3	20	Apparently normal. Littermate of 7, identical course of recovery
9	F	8	3 days	Comatose throughout	Lies on side, opisthotonus. No righting reflexes			No recovery from coma
10	F	8	4 days	Comatose throughout	Lies on side. No righting reflexes			No recovery from coma
11	M	8	5 days	Comatose throughout	Lies on side. No righting reflexes			No recovery from coma
12	M	8	2 days	Comatose throughout	Lies on side. No righting reflexes			No recovery from coma
13	M	8	2½ months	Comatose throughout	Can sit up after a week. Never stands			Permanent defects: (see text)
14	F	10	6 days	Comatose throughout	No righting reflexes			No recovery from coma. Drinks milk when mouth is placed in it. Complex responses in elimination
15	F	11½	2 hours	Comatose	Lies on side. No righting reflexes			Continues to gasp until death. Rectal temperature rises to 110°F. Pin point pupils. Spinal shock

Lapping was rapid and vigorous at first, but after ingestion of some quantity of milk, gradually ceased, after which the head was withdrawn. The

comatose dog lifted the hind leg in urination. Elevation of the tail, flexion of the thighs and pelvis, running movements of the hind legs, vocalization and sometimes rhythmic chewing and lapping accompanied defecation.

Respiration was usually slow and deep, and there was no sign of a Cheyne-Stokes rhythm. There was a definite bradycardia, with a pulse rate as low as 63 per minute and a marked respiratory arrhythmia of the heart. Body temperature was normal but shivering occurred at environmental temperatures of 80°F.

4. Period of apathy and severe ataxia. Dogs which survived arrest of brain blood flow for 8 minutes or longer never recovered to this stage. In animals which were subjected to brain stasis of 6 minutes or less, a period of dullness and apathy intervened between the period of coma and recovery of function of the higher centers (table 1).

The earliest sign of recovery from coma was the restoration of vestibular function and righting reflexes, which enabled the animal to turn from his side to his abdomen, although still unable to stand or walk. Flexor rigidity was rapidly disappearing. In sitting up, he frequently stepped on the dorsum of the forefoot. The forelimbs appeared to be much stronger and more active than the hindlimbs, and were used in crawling on the abdomen.

During this early period of returning brain function, spontaneous movement was at a minimum, consisting of cleaning and licking, sitting up, turning the head and attempts to crawl. As earlier, milk was lapped. If now the food was moved beyond reach, the hungry dog extended his head toward it but made no attempt to crawl.

The emotional behavior of the animal was abnormal. Visual or auditory stimuli elicited a type of "sham rage". The presence of this response was a quickly passing phase of recovery, since less than a day later it was impossible to evoke the pattern.

The next stage of recovery was characterized by return of the ability to stand and walk and increased spontaneous activity. At first the dog stood on a broad base, constantly shifting his position and showing severe ataxia, which was similar to that seen in cerebellar dysfunction in man. There was at this stage no evident rigidity, paralysis or defect in conscious proprioception. The tendency to step on the dorsum of the forefoot persisted.

While cerebral function had improved by this time, there were still evidences of depression of the higher centers. Although the dog now recognized food and fed himself, there was no response to other animals. He was unable to perform previously learned tricks and was still lethargic.

5. Period of residual ataxia. At this stage, the behavior of the animal could not be distinguished from the normal by simple observation in the

laboratory. He regained the ability to perform tricks without relearning. The dog fell infrequently now, no longer stepping on the dorsum of the forefoot. He still suffered from an ataxic gait, this being the only residual deficiency. Coördination of movement gradually improved and later a deficiency was evident only when the animal was excited and very active or when the floor was wet.

6. Recovery. All dogs surviving periods of arrest of the brain circulation of 6 minutes or less eventually recovered completely, as far as we could determine without the employment of special technique for study of the higher cerebral functions. Such animals have been kept under observation in the laboratory for several months, during which time they have behaved normally.

C. Permanent brain damage resulting from 8 minutes of arrest of blood flow. One dog which was subjected to arrest of the brain circulation for 8 minutes was kept alive for $2\frac{1}{2}$ months, during which time only slight recovery of brain function was observed (dog 13, table 1). He recovered to a stage between quiescent coma and apathy. Auditory and visual reflexes, sensation, feeding reactions, and cardiac rhythm remained as described in the section above on quiescent coma. However, this animal regained some vestibular and righting reflexes. Spontaneous movements were rare and consisted of sitting up, turning the head, licking chops and licking the fur, the latter being insufficient to keep the body clean. He regained no power of locomotion or vocalization, and emotional responses were not elicited. When the animal was supported in the upright horizontal position with the limbs free, extensor spastic rigidity became apparent. This spasticity was more marked in the forelimbs than in the hindlimbs, while there was no extensor rigidity of the neck, and the tail was markedly flexed, curled under the abdomen and rigid. Passive rotation of the head usually failed to alter the posture of the limbs. The placing reaction to touching the dorsum of the foot to the edge of the table was absent in the forelimbs but present in the hindlimbs. When the dog was placed on his side or back, a flexor rigidity similar to that seen in quiescent coma became evident.

Discussion. The marked functional depression of the cerebellum by arrest of the brain circulation, as indicated by the persistence of ataxia long after the restoration of cerebral function, suggests that the cerebellar neurons may be the most sensitive to anoxia. This view is supported by the fact that the cerebellar cortex shows the greatest oxygen consumption (7), and that action potentials disappear first in this region during brain anemia (8). Observations in humans subjected to anoxia (9) and carbon monoxide (10) also confirm the greater susceptibility of the cerebellum. In patients resuscitated after hanging (11), cerebellar symptoms persisted after recovery of cerebral function. Microscopic studies of the brain in

dogs (12) and cats (13) reveal that the Purkinje cells of the cerebellar cortex are most susceptible to arrest of blood flow. Since the Purkinje cell is an essential link in the neuron chain of cerebellar activity, any damage to these neurons is immediately reflected in loss of function.

Many of the phenomena observed following brain stasis have been described in decorticate animals (14): Stepping on the dorsum of the forefoot, infrequent restricted spontaneous movements, loss of placing reactions, inadequate cleaning responses, hyperirritability of the shivering mechanism (15), retention of taste and of complex responses of feeding and elimination. The extensor rigidity which appeared when the dog was supported in the upright position with the legs free is similar to "decorticate rigidity" (14, 16), although spasticity was not observed in the dorsal decubitus in our experiments. The blindness and anosmia were probably cortical in origin. In contrast to decorticate preparations, our animals lacked auditory reflexes.

The marked flexor statue-like rigidity observed during the period of quiescent coma is suggestive of altered function of the basal ganglia. This rigidity has many of the characteristics of basal ganglion disease in the human, being "soft", flexor in type, showing resistance that is equal throughout the passive manipulation with no lengthening or shortening reactions, involving only the more proximal joints of the limb and being uninfluenced by rotation of the head or changes in the position of the head in space. When the limb is passively extended, it returns only slowly to the original flexed position. In comatose patients resuscitated after hanging, flexor rigidity attributed to loss of function of the globus pallidus has been observed (11, 17). It is well known that the basal ganglia are very susceptible to anoxia in man, as seen in carbon monoxide poisoning and arteriosclerosis.

A number of other findings in dogs following brain stasis are referable to dysfunction of the brain stem: loss of auditory reflexes, loss of emotional reactions and vocalization, loss of the ability to stand and walk, loss of vestibular and righting reflexes. It is of interest to note that dogs sacrificed soon after acute carbon monoxide asphyxia show histological changes in the vestibular nuclei while animals surviving the acute asphyxia for several months have normal vestibular nuclei (18). Another characteristic finding, the bradycardia and respiratory arrhythmia observed in comatose dogs, can perhaps be explained as a release of the vagus center from higher inhibition (3).

The period of hyperactive coma, characterized by spontaneous coördinated running movements and vocalization, has been observed following brain anemia in cats (1) and dogs (19). In man, running movements, inarticulate vocalization and reflex hyperexcitability have been described in the early period of recovery from strangulation (11, 20) and from cardiac

arrest (21). Similar running movements occur during recovery from barbiturate anaesthesia.

Various workers (1, 2, 22) have reported the occurrence of epileptiform convulsions, sometimes developing into fatal status epilepticus, in cats following arrest of the brain circulation. On the other hand, in our own experiments and those of others with dogs (19), as well as in clinical studies of arrest of the circulation to the human brain (11, 20, 21), convulsions did not occur following restoration of blood flow. In the single instance in our experiments in which epileptiform convulsions appeared, the brain stasis had been incomplete. While arrest of blood flow was incomplete in some experiments with cats (2, 22), this was apparently not the case in others (1) and one must resort to a species difference to explain the discrepancy. This problem has been discussed from the clinical point of view by Cobb (23), who comes to the conclusion that partial anemia is much more likely to produce convulsions than complete anemia of brain tissue.

The statement which was made previously that dogs which survived complete arrest of the cephalic circulation of 6 minutes' duration recovered apparently completely must be qualified, since the examination of cerebral function was limited to observation in the laboratory and tests of ability to retain simple tricks learned previously and to learn such responses anew. The possibility of permanent disturbance of the higher functions of the cerebral cortex cannot be disregarded, since Andreyev (24) has demonstrated, by the method of conditioned reflexes, deficiencies in higher functions in dogs which appeared normal in ordinary examination following brain anemia.

The similarity of the course and detailed findings in patients recovering from acute strangulation (11, 20) and of the effects of arrest of the brain circulation in our experimental animals is very striking. These patients show the period of hyperactive coma with running movements and inarticulate vocalization, the early extensor rigidity and opisthotonus, the later flexor rigidity and extrapyramidal signs, the return of cerebral function with disorientation and clouding of consciousness, the late cerebellar symptoms and the eventual complete recovery. This similarity leads one to hope that the investigation of the effects of arrest of the brain circulation in animals may prove to be of value in the understanding of circulatory disorders in the human brain.

SUMMARY AND CONCLUSIONS

1. By means of a new technique, sudden complete arrest of blood flow in the brain has been produced in dogs.

2. The course of recovery is qualitatively similar but differs quantitatively in different dogs depending on the duration of the arrest of the cephalic circulation. The course of recovery may be conveniently divided

into six stages: a, period of early return of function; b, period of hyperactive coma; c, period of quiescent coma; d, period of apathy and severe ataxia; e, period of residual ataxia; f, recovery.

3. Complete arrest of the brain circulation results in disappearance of the corneal reflex in 10 seconds and of respiratory function in 20 to 30 seconds.

4. Arrest of the cephalic circulation causes spinal shock, which passes off gradually after blood flow is restored.

5. Arrest of the brain circulation for 6 minutes or less results in apparently complete recovery of function, while brain stasis for 8 minutes or longer results in permanent severe damage to the brain. The effects of cephalic stasis are the same for each period of stasis investigated regardless of the sex, weight or breed of the experimental animals.

6. Relatively soon after restoration of function of the vital centers, a period of hyperactivity and reflex hyperirritability ensues which persists for several hours. This period is characterized by vigorous, rapid, coordinated running movements of all 4 limbs carried out with the animal lying on his side in coma. No epileptiform convulsions have ever been observed following complete arrest of cephalic blood flow.

7. Coma may persist for as long as 24 hours after only 4 minutes of arrest of the brain circulation. The coma is characterized by loss of auditory reflexes, vestibular and other righting reflexes in addition to loss of function of the cerebral cortex. Early in coma a moderate spasticity is evident, while later a flexor rigidity suggestive of involvement of the basal ganglia becomes apparent.

8. Following coma, there is a transition period lasting several days which is characterized by gradually improving function of the cerebral cortex and of the righting mechanisms, as well as by severe ataxia of cerebellar type.

9. In dogs which recover consciousness, the most persistent neurological dysfunction is ataxia. This gradually disappears, leaving an animal which cannot be distinguished by our methods of examination from the normal.

10. The permanently defective brain function produced by 8 minutes of arrest of brain blood flow consists of loss of function of the cerebral cortex, loss of auditory reflexes, of the ability to stand and walk, of emotional reactions and vocalization, as well as dysfunction suggestive of striatal involvement.

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A QUANTITATIVE METHOD FOR THE MEASUREMENT OF THE RATE OF WATER LOSS FROM SMALL AREAS, WITH RESULTS FOR FINGER TIP, TOE TIP AND POSTERO-SUPERIOR PORTION OF THE PINNA OF NORMAL RESTING ADULTS

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Most of the methods previously described for the measurement of the rate of sweating from small areas of the human skin have been qualitative (1, 2, 3, 4, 5). A number of quantitative methods have been advocated (5, 6, 7, 8). We found that those that involve the use of an absorbent material, such as filter paper (8) or cloth (6) are inaccurate and non-reproducible. Kuno (5) studied the rate of sweating by using U-tubes filled with calcium chloride to trap the moisture picked up by dry air that had been allowed to flow over a small area of skin. In attempts to use this method it was found that the removal of moisture by calcium chloride U-tubes was not complete at the rate of flow of air necessary to remove the moisture from the skin. Even 1500 grams of finely divided calcium chloride enclosed in a glass tube four feet long did not adequately dry such a stream of air or oxygen. Greuer and Peukert (7) described a method for ascertaining the amount of moisture lost from human skin by measuring variations in the resistance of a semi-conductor. In their method a known area of skin was covered with a shallow chamber roofed by a sodium chloride crystal. The rate of change in resistance of the crystal produced by the water evaporating from the skin and being deposited upon its surface was used as an index of the rate of the elimination of water. Lack of satisfactory standardization of the method made it inadvisable to use it in its present form. A more complete review of the various procedures that have been employed can be found in the publications of Kuno (5), McSwiney (6) and Greuer and Peukert (7). Because of the inadequacy of these methods it became desirable to develop a more accurate procedure. This report concerns itself with a description of, and the results obtained with, the method evolved.

Materials and method. The apparatus can be divided into three parts: 1, metal cups enclosing the part studied; 2, aluminum coils for the collection of water; 3, a system for providing a stream of dry oxygen.

[1] Commonwealth Fund Fellow.

1. The chambers for enclosing the finger tips and toe tips were constructed of brass sheeting (0.003 inch thick), cut, shaped and soldered together to form cylinders (diameter about 3 cm.; height, about 4 cm.) (fig. 1 A). The chambers were made of metal instead of cellulose acetate since this substance permits a slow diffusion of water. An opening was made in one end (the proximal end) of the cylinder for the entrance of the part to be studied. Four radial brass tubes (inlets) with an inside diameter of approximately 2 mm. were soldered into the circular wall of the cylinder near the proximal end. A fifth brass tube (outlet) with an inside diameter of approximately 3.5 cm. was soldered to the center of the distal end of the cylinder. A short piece of flexible rubber tubing was fitted over the proximal end of the cylinder. To make an air-tight seal this tubing was

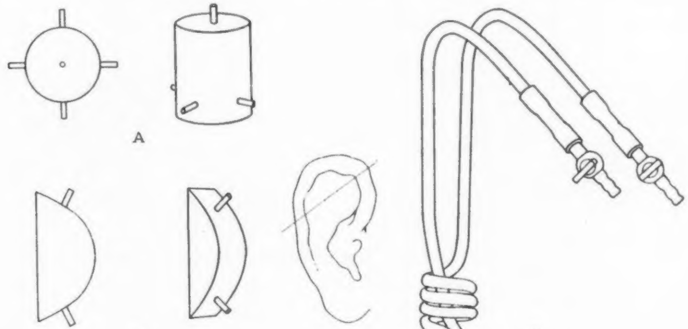

Fig. 1. A—Brass cylinder for enclosing finger tip or toe tip. B—Brass hemicylinder for enclosing postero-superior portion of pinna. C—Aluminum coil for collecting water.

chosen to fit closely to, but without constricting, the part inserted for study.

The chamber for enclosing the postero-superior portion of the pinna was also constructed of brass sheeting but was a hemi-cylinder (height about 1.5 cm.; radius about 2.5 cm.) (fig. 1 B). A wide more or less crescent-shaped opening was cut into the flat surface (base) and closed by a rubber membrane. In this an opening was cut, so shaped as to conform accurately to the shape of the pinna lying in the opening. A brass tube (inside diameter approximately 3 mm.) was soldered to each end of the curved wall. One tube served as an afferent and the other as an efferent.

2. A number of aluminum coils (fig. 1 C) for the collection of water was constructed of approximately 1 meter of aluminum tubing (outside diameter 4.8 mm.; inside diameter 3.2 mm.). Each end of the coils was guarded by a metal stopcock. The coils were made to weigh 50 grams to facilitate successive weighings.

3. A stream of oxygen flowing from a tank passed through rubber tubing to an aluminum coil (fig. 2). The coil was placed in a thermos bottle containing a freezing mixture of ethyl alcohol and CO_2 snow. In this coil the oxygen was dried. From the coil it passed in an aluminum tube at least 12 feet long and a four-way distributor into the four inlets of a brass cylinder. From the brass chamber, the oxygen, now carrying sweat, proceeded by rubber and aluminum tubing to a second aluminum coil. This coil was also placed in a thermos bottle containing a freezing mixture of ethyl alcohol and CO_2 snow. By means of a system of stopcocks and

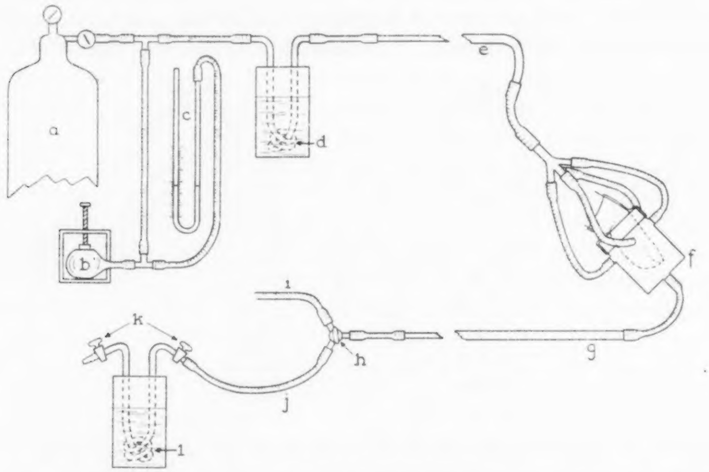

Fig. 2. A complete diagram of the apparatus. *a*, oxygen supply; *b*, pressure bulb controlled by screw clamp; *c*, water manometer; *d*, aluminum coil for drying the oxygen; *e*, aluminum tubing conducting dry oxygen to part; *f*, metal cylinder enclosing part; *g*, aluminum tubing conducting water laden oxygen from part; *h*, 3-way stopcock permitting distribution of oxygen through rubber tubing, *i* or *j*, to alternate water collecting coils; *l*, aluminum water collecting coil with stopcocks, *k*.

rubber tubing it was possible to change the flow to a number of aluminum coils in succession, in order to collect sweat for varying periods of time.

To ascertain the accuracy of the method a brass chamber containing a few drops of water was substituted for the usual cylinder. The air in the chamber was displaced with oxygen and its initial weight obtained. Dry oxygen was passed through the rubber and aluminum tubing for 15 minutes to insure dryness of the system. This oxygen was then permitted to flow through the brass chamber and a weighed aluminum coil immersed in the freezing solution. The water vapor carried from the brass chamber was

deposited in the aluminum coil and the oxygen, having deposited its water, was allowed to escape. After a few minutes the brass chamber was removed. The lengths of tubing which had been connected to the inlet and outlet tubes of the chamber were connected to each other and dried with dry oxygen for 15 minutes. The stopcocks of the aluminum coil were then closed to prevent the escape of water when the coil returned to room

TABLE 1

Data showing the results of the standardization of the method

SAMPLE NUMBER	WATER INTRODUCED INTO THE SYSTEM	WATER COLLECTED BY APPARATUS	DIFFERENCE	ERROR	DIFFERENCE AFTER CORRECTION OF 0.4 MGM.	ERROR AFTER CORRECTION
	mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*
1	69.6	71.1	+1.5	+2.15	+1.9	+2.7
2	49.9	49.8	−0.1	−0.20	+0.3	+0.6
3	47.4	46.1	−1.3	−2.74	−0.8	−1.7
4	31.8	31.1	−0.7	−2.20	−0.3	−0.9
5	31.2	30.6	−0.6	−1.92	−0.2	−0.6
6	28.0	27.3	−0.7	−2.50	−0.3	−1.1
7	21.4	21.1	−0.3	−1.40	+0.1	+0.5
8	20.4	20.6	+0.2	+0.98	+0.6	+2.9
9	20.3	19.9	−0.4	−1.97	0.0	0.0
10	17.3	17.9	+0.6	+3.46	+0.9	+5.2
11	15.6	15.5	−0.1	−0.64	+0.3	+1.9
12	14.2	13.5	−0.7	−4.93	−0.3	−2.1
13	14.0	13.2	−0.8	−5.71	−0.4	−2.9
14	12.1	11.5	−0.6	−4.95	−0.2	−1.6
15	12.0	12.1	+0.1	+0.83	+0.5	+4.2
16	12.0	11.4	−0.6	−5.00	−0.2	−1.6
17	11.7	10.8	−0.9	−7.69	−0.5	−4.3
18	7.5	6.8	−0.7	−9.30	−0.3	−4.0
19	7.2	6.7	−0.5	−6.99	−0.1	−1.4
20	5.3	5.1	−0.2	−3.70	+0.2	+3.8
21	4.3	3.8	−0.5	−1.16	−0.1	−2.3
22	3.6	2.9	−0.7	−19.40	−0.3	−8.3
23	3.4	2.7	−0.7	−20.50	−0.3	−8.8
24	3.2	2.8	−0.4	−12.50	0.0	0.0
25	2.6	2.2	−0.4	−15.30	0.0	0.0
Mean.......	18.6	18.2	−0.4			2.6

temperature. The brass chamber and the aluminum coil were both weighed a second time to learn the amount of water which had been lost from the former and the amount gained by the latter. This procedure was repeated for 25 separate measurements (table 1).

The mean loss of water from the brass chambers was 18.6 mgm. and the mean amount of water picked up in the aluminum collecting coils was 18.2

mgm., an error of −0.4 mgm. or −2.2 per cent (table 1). The variation from this mean error was small. Such an error, insignificant for large amounts, became increasingly important as the amount of water deposited decreased. Since the error was about 0.4 mgm. and since we were not in position to learn its source, we corrected our results arbitrarily by the addition of this amount. The correction is applicable in the 15 subjects.

To measure the rate of perspiration, fingers, toes or ears were sealed in their brass cylinders with rubber cement. Since three parts were studied simultaneously, three separate streams of dry oxygen were provided. Leaks were detected by the use of a water manometer. The oxygen flow was adjusted to 300 to 500 cc. per minute in each chamber, pressure not exceeding 3.5 cm. of water. For the first 30 minutes, oxygen being used to dry the systems was allowed to escape. To collect and measure the water eliminated, the oxygen was made to pass through the aluminum coils immersed in the freezing mixture. After a certain time the flow having passed through one group of aluminum coils was directed through a second group. Flow through a succession of coils was carried out for 15 minute intervals for a total of 60 to 90 minutes.

Certain precautions in weighing the coils were taken: 1. To insure uniform dryness before use the inside of the coils was dried by passing room air through them and the outside by blowing room air over them. The coils were then filled with dry oxygen at atmospheric pressure before being weighed. 2. After the collection of the water, the oxygen in the coils having attained room temperature was brought to atmospheric pressure by opening one of the stopcocks momentarily to allow the escape of the excess of oxygen.

The flow of the dry oxygen through the brass chambers did not affect their temperature materially as measured by thermocouples placed within the chambers.

Because of variations in the size of the parts studied, measurements of the surface area of the finger tip, toe tip and postero-superior portion of the pinna were made using methods previously described (9, 10, 11). The formula given by Isbell (9) for measuring the surface area of the finger tip from its volume was found applicable to the toe tip with a maximum error of 3 per cent. The finger tip as used in these studies is defined as that portion of the finger distal to a plane passing through the distal major dorsal and palmar skin creases. The toe tip is that portion of the toe distal to a plane passing through the distal major dorsal and plantar skin creases. The postero-superior portion of the pinna lies above a plane passing at right angles to the lateral surface of the pinna and slightly postero-superior to the portion of the pinna joining the scalp (see fig. 1).

Results. Measurements of the rate of water eliminated from small areas of skin were made of 15 normal white adults (6 males and 9 females)

varying in age from 22 to 52 years. The subjects were studied at various times of the day while resting in bed and covered to satisfy each individual's comfort. The atmosphere of the room was controlled to maintain a temperature of 75°F. ±1 and a relative humidity of 50 per cent ±3. The parts for study were adjusted to the level of the heart. The subjects rested for an hour before collections were started. In 7 of the subjects the measurements were repeated after an interval of several days to weeks.

The mean amount of water collected from the right index finger tip of the resting subject was 1.86 mgm. per square centimeter per 15 minutes, the variations ranging from 3.82 to 0.81. The mean value for the right second toe tip was 1.18 mgm. per square centimeter per 15 minutes, the variations ranging from 2.16 to 0.52. The values for the postero-superior portion of the pinna of the right ear were found to possess a mean of 0.48

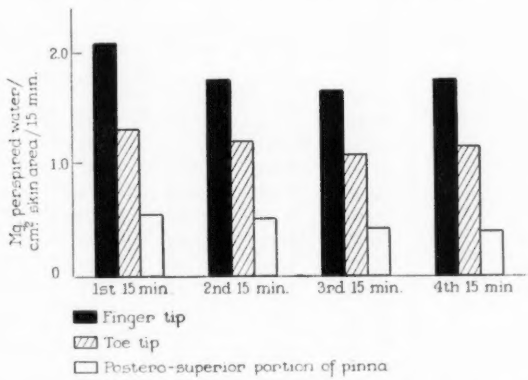

Fig. 3. Proportional rates of water perspired by finger tip, toe tip and pinna during four successive 15-minute periods.

mgm. per square centimeter per 15 minutes, the variations ranging from 0.69 to 0.29. The rate of the loss of water was greater from the finger tip than from the other two parts studied. The ratio of the mean rates of the elimination of water of the finger tips to toe tips was approximately three to two; of finger tips to postero-superior portion of the pinnae, approximately four to one. In two subjects the finger tip to toe tip ratio deviated markedly, being almost one to one. In most of the measurements it was found that the rate of the loss of water in the first 15 minutes was slightly greater than in the succeeding fifteen minute periods when there was a tendency to reach a constant level (fig. 3).

Discussion. The method employed in these observations is open to certain objections; 1, because the atmosphere surrounding the parts studied was different from that surrounding the rest of the body; 2, because the

relative humidity of the room being 50 per cent, while that in the brass chambers was much less, may have tended to increase the rate of evaporation from the surface of the skin. The degree to which the transpiration of water and the activity of sweat glands were influenced by these factors is unknown. The nature of the gas surrounding the parts may have influenced the results but the degree was probably insignificant. In spite of these criticisms the method offers certain advantages. It is simple, accurate, and does not disturb the subject after the parts have been enclosed in the brass chambers. Controlling the flow of oxygen, turning the stopcocks, changing the collecting coils, weighing and drying the coils, and per-

TABLE 2

Rate of sweating (mgm./sq.cm. surface area/15 min.) in the right index finger tip of 13 normal white resting adults

SUBJECT NUMBER	AGE	SEX	SURFACE AREA FINGER TIP	FIRST 15 MINUTES	SECOND 15 MINUTES	THIRD 15 MINUTES	FOURTH 15 MINUTES	MEAN FOR 15 MINUTES
	years		*sq. cm.*					
1	30	M	11.64	2.56	2.27	1.72	1.63	2.05
2	40	M	10.64	2.27	1.84	2.07	1.97	2.04
3	33	M	11.47	1.83	1.31	1.31	1.74	1.55
4	27	M	12.06	2.67	1.92	1.44	1.41	1.86
5	28	M	12.94	2.26	3.82	2.13	1.74	2.49
6	39	F	9.81	2.75	2.08	1.78	2.01	2.16
7	30	F	8.77	1.38	1.37	1.40	1.74	1.47
8	40	F	9.04	1.64	1.97	2.01	2.03	1.91
9	23	F	10.25	1.95	1.83	1.88	1.87	1.88
10	52	F	10.17	1.01	0.93	0.81	1.12	0.97
11	25	F	10.51	2.81	2.03	1.51		2.12
12	30	F	8.54	1.79	1.55	1.43	1.43	1.55
13	50	F	10.58	2.16	1.85	2.15	2.40	2.14
Mean				2.08	1.75	1.66	1.76	1.86
Max.				2.81	3.82	2.13	2.40	2.49
Min.				1.01	0.93	0.81	1.12	0.97

forming the other necessary manipulations can be, and were, done far removed from the subject's bed. By modifying the size and shape of the various parts of the system the method can be used to study the rate of elimination of water from many normal or diseased surfaces.

The differences in the rate of loss of water from the parts studied were marked. The rate was two-thirds as rapid in the toe tip as in the finger tip and one-quarter as rapid in the postero-superior portion of the pinna as in the finger tip. The reason for these differences may be physiologic or anatomic or both. Since there are no satisfactory anatomical data (5, 12) on the number of sweat glands in the areas studied it is impossible to decide what the reasons are which account for the difference.

There are marked variations in the rate of water loss in different individuals (tables 2, 3 and 4). The rate of elimination of water from the

TABLE 3

Rate of sweating (mgm./sq.cm. surface area/15 min.) in the right second toe tip of 14 normal white resting adults

SUBJECT NUMBER	AGE	SEX	SURFACE AREA TOE TIP	FIRST 15 MINUTES	SECOND 15 MINUTES	THIRD 15 MINUTES	FOURTH 15 MINUTES	MEAN FOR 15 MINUTES	RATE OF SWEATING AS PER CENT OF FINGER TIP RATE
	years		*sq. cm.*						
1	30	M	10.99	1.09	0.79	0.69	0.70	0.82	39
2	40	M	10.70	1.48	1.40	0.97	1.06	1.23	58
3	33	M	10.51	1.41	1.41	1.17	1.35	1.34	90
4	27	M	9.97	1.15	1.18	0.82	0.91	1.02	61
5	28	M	12.12	1.56	2.16	1.58	1.47	1.69	68
6	39	F	10.92	1.62	1.09	0.89	1.09	1.17	52
7	30	F	8.81	0.81	0.81	0.52	0.96	0.78	51
8	40	F	10.50	1.00	0.84	0.89		0.91	43
9	33	F	11.23	1.02	1.21	0.90	0.94	1.02	55
10	52	F	8.67	0.82	0.89	0.82	0.91	0.86	92
12	30	F	8.33	1.13	0.90	1.12	1.07	1.06	70
13	50	F	9.17	1.35	1.30	1.29	1.49	1.33	64
14	22	F	8.55	2.08	1.52	1.73	1.72	1.76	
15	30	M	10.79	1.82	1.13	1.65	1.30	1.48	
Mean				1.31	1.19	1.07	1.15	1.18	
Max.				2.08	2.16	1.73	1.72	1.76	
Min.				0.81	0.79	0.52	0.70	0.78	

TABLE 4

Rate of sweating (mgm./sq.cm. surface area/15 min.) in the postero-superior portion of the pinna of 5 normal white resting adults

SUBJECT NUMBER	AGE	SEX	SURFACE AREA OF PORTION OF PINNA	FIRST 15 MINUTES	SECOND 15 MINUTES	THIRD 15 MINUTES	FOURTH 15 MINUTES	MEAN	RATE AS PER CENT OF FINGER TIP RATE
	years		*sq. cm.*						
1	30	M	13.21	0.64	0.60	0.57	0.50	0.58	30
2	40	M	13.86	0.66	0.69	0.42	0.40	0.54	26
3	33	M	13.02	0.50	0.44	0.41	0.42	0.44	29
4	27	M	13.28	0.41	0.38	0.41	0.30	0.38	23
13	50	F	13.60	0.49	0.41	0.29	0.34	0.39	16
Mean				0.54	0.50	0.42	0.39	0.48	
Max.				0.66	0.69	0.57	0.50	0.58	
Min.				0.41	0.38	0.29	0.30	0.38	

finger tip of subject number 2, for instance, was almost twice as rapid as from subject number 10. Although the rate of the elimination of water

varied within the same individual from time to time the level remained fairly constant. The rates are apparently correlated with the emotional type of the subject. Phlegmatic subjects lost water less rapidly than excitable ones.

SUMMARY

A method is described for measuring the rate of water loss from small surfaces. The method consists in passing dry oxygen through chambers covering the surfaces and then conducting the moisture-containing oxygen through cold aluminum coils. From the difference in weight of the coils before and after the passage of the oxygen, the amount of water lost is learned. The method is accurate to 2.6 per cent. This error can, however, reach 9 per cent when less than 6 mgm. of water are measured, but such low values were not encountered.

The rate of the elimination of water was studied from the right index finger tip, right second toe tip and postero-superior portion of the right pinna of 15 white, normal, resting adult subjects. The mean rate of water loss was found to be 1.86 mgm. per square centimeter per 15 minutes for the finger tips, 1.18 mgm. for the toe tips and 0.48 mgm. for the pinnae. The rate of water loss in the toe tips was approximately two-thirds as rapid as in the finger tips and the rate for the pinnae was only one-quarter as rapid as that for the finger tips.

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THE LACTIC ACID MECHANISM AND CERTAIN PROPERTIES OF THE BLOOD IN RELATION TO TRAINING

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In work where the rate of energy expenditure exceeds the capacity for oxidative processes, a considerable portion of the energy supplied anaerobically depends on the "lactic acid mechanism" for contracting an O_2 debt, an expression applied by Margaria, Edwards and Dill (1933). In strenuous athletics the lactic acid mechanism as a source of anaerobic energy in muscular contraction is of major importance, particularly in maximal effort of short duration. The observations of Herxheimer (1924) that training increases the alveolar CO_2 tension, and of Wissing (1926), Rehberg and Wisseman (1927), Ewig (1928) and Full and Herxheimer (1926) that training increases the alkaline reserve indicate that training may increase a man's tolerance for lactic acid in severe anaerobic work. Dennig (1937) has found that work capacity is increased when the alkaline reserve is raised by ingesting mixtures of sodium citrate, sodium bicarbonate and potassium citrate. Robinson, Edwards and Dill (1937) found the basal alkaline reserve in 5 champion mile runners to be the same as in non-athletes. Thörner (1932) found that training had no effect upon the alkaline reserve of dogs. Recent unpublished observations by the present authors made on 15 highly trained college runners under basal conditions show an average alkaline reserve of 21.5 mM. per liter of blood at 40 mm. Hg pCO_2. This average is no higher than the generally accepted averages for non-athletes and every individual athlete was within the normal range. In basal alveolar air samples from these college athletes and the 5 champion runners we found a mean CO_2 tension of 41.9 mm. Hg with the highest value 45.

The present study is designed to determine the effects of training upon the lactic acid mechanism in work and its relation to certain properties and constituents of the blood; the alkaline reserve has received particular attention because of the above mentioned variance in results. As subjects 9 non-athletic college students, ages 18 to 22, were chosen from 40 applicants who went through preliminary tests on the treadmill. The men chosen possessed varied athletic ability from the poorest to the best of the

40 applicants. To insure coöperation they were paid for their time. The training was continued for a period of 6 months and consisted of a carefully supervised running program with four workouts on the track each week during the first month and thereafter workouts on Tuesday, Wednesday and Thursday of each week with a time trial every Saturday to measure progress. The Saturday trials were at $\frac{1}{2}$, $\frac{3}{4}$, 1 and $1\frac{1}{2}$ miles in rotation. About once a month a mid-week trial in a sprint was held to test speed. The training program each week was planned to give the men the best preparation for the coming trial, with over-distance running one day and pace work and speed work on the other two days. The men performed as much running during training as their legs could stand without developing handicapping soreness. Gymnasium work was included each day for general conditioning. An example of the progress made in training was the consistent lowering of the men's time for the mile run: in December the 9 men averaged 6 minutes and 24 seconds on the mile as compared with 5 minutes and 15 seconds the following April.

Observations in the laboratory were made on the men before training started and at regular intervals during the training period. The work tests in the laboratory were performed on a motor driven treadmill and consisted of: 1, a standard 15-minute walk at 5.6 km. per hour on an 8.6 per cent grade with finger blood being drawn for lactate and sugar after 10 minutes of walking when the subjects had attained a steady state; 2, a 10-minute run on the level at a moderate pace which was 12.9 km. an hour for 7 of the men and 14 km. for the two best runners; blood for analysis was drawn from an arm vein 5 minutes after the end of the run; 3, a run severe enough to exhaust the men in 3 to 5 minutes. During the training period each time a man became able to complete 5 minutes of the exhausting run the grade or speed or both were increased for him in the next test in an attempt to keep the work just severe enough to exhaust him in 4 to 5 minutes, his goal being to complete the 5 minutes. As in the 10-minute run blood was drawn 5 minutes after the exhausting run. (We have used the concentration of lactic acid in venous blood drawn 5 minutes after stopping these runs to indicate the extent to which the lactic acid mechanism has been brought into action: according to Margaria, Edwards and Dill (1933) the lactate at that time has become uniformly distributed between tissues and blood. Evidence of Hill (1928), Evans (1930) and Newman (1938) support this assumption.) All repetitions of the 15 minute walk and the 10-minute or moderate run for each man were at the same intensity as in the original tests. About once a month each man repeated a 5 minute run on the treadmill at the same speed and grade as the run which was originally exhausting for him. Basal alveolar air and venous blood samples were drawn with the men in the recumbent position at the end of basal metabolism determinations.

Blood lactate was determined by the method of Edwards (1938), blood sugar by the method of Folin and Malmros (1928) modified for the photoelectric colorimeter, and plasma protein by micro-Kjeldahl analysis. HbO_2 capacity and alkaline reserve were determined by equilibration of blood with O_2 and CO_2 pressures of 200 and 40 mm. Hg respectively at 37°C. as described by Dill in Henderson's book (1928). The blood gases were

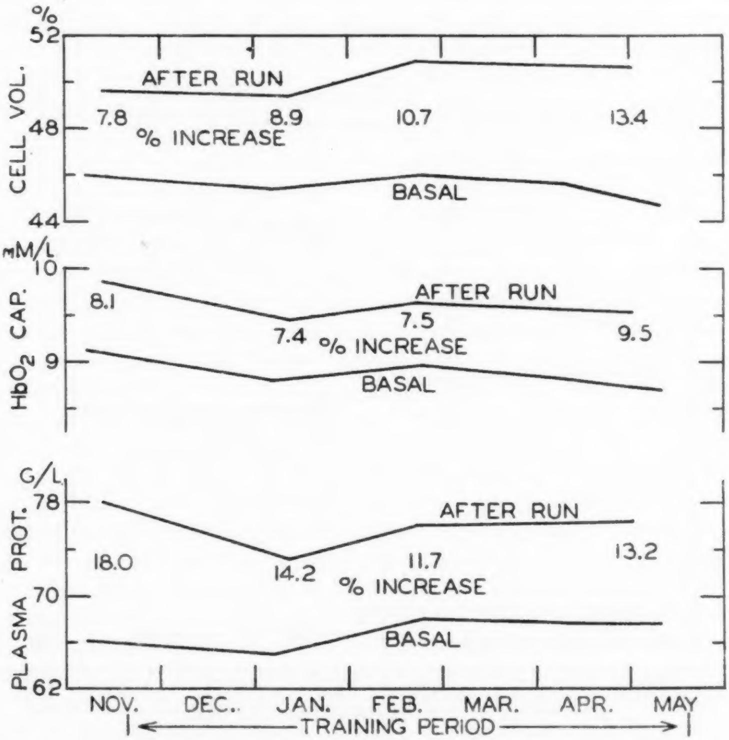

Fig. 1. The effects of training on the blood of 9 men. Mean values in the basal state and 5 minutes after exhausting work.

analyzed in the Van Slyke apparatus. The alkaline reserve as used in this paper is the CO_2 content of oxygenated whole blood at 40 mm. Hg CO_2 tension with temperature 37°C. Alveolar air samples were collected in the basal state as described by Bock and Field (1924) and during work by the method of Henderson and Haggard (1925), analysis being made with the Haldane apparatus.

The basal state. Figure 1 reveals that training had no significant effect

upon the HbO_2 capacity or cell volume of the blood nor upon the plasma protein in the basal state. The mean values of these constituents are all very close to generally accepted standards. Certainly there is no tendency for any of them to increase with training. Variations of the means of the 3 components tend to coincide with each other. Variability of individual values is about the same in the successive determinations. For example, the extreme basal HbO_2 capacities in November were 7.9 and 9.6 mM. per

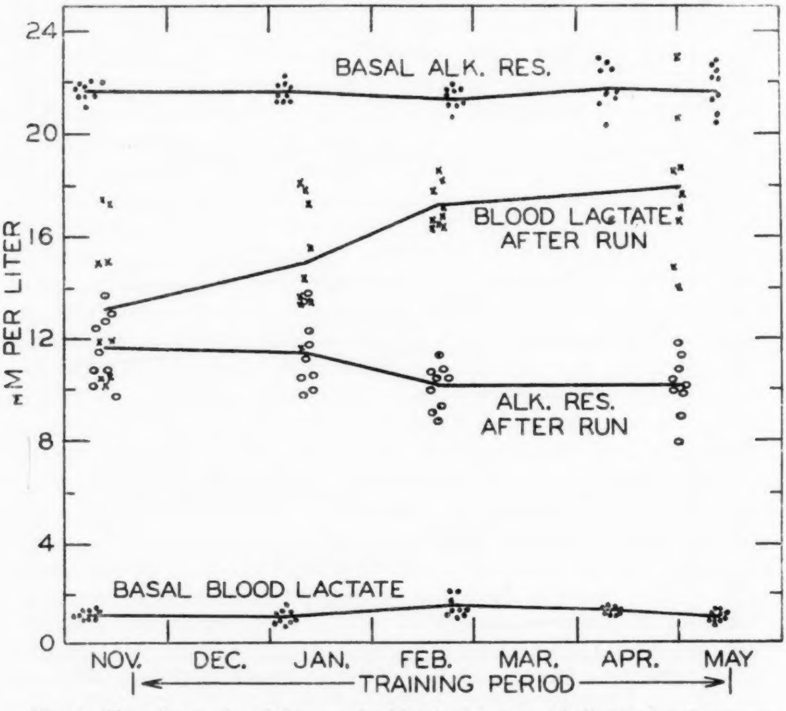

Fig. 2. The effects of training on the blood of 9 men. Individual and average values of alkaline reserve and lactic acid in the basal state and 5 minutes after exhausting work.

liter as compared with 7.9 and 9.5 by the same individuals in May. Figure 2 includes scatter diagrams of basal blood lactate and alkaline reserve. Lactate shows no change of the average or of the variability resulting from the training. On the average the alkaline reserve remained unchanged but it showed a greater variability in the later months of training. The values are all well within the usual limits for untrained people according to the data of Hurxthal, Bock, Talbott and Dill (1929), Dill,

Edwards and Consolazio (1937), Robinson (1938) and of Dill, Wilson, Hall and Robinson (1940). Our data thus fail to show an increase in alkaline reserve in training as was reported by the European workers cited above. Dietary effects on the alkaline reserve should be considered as a possible explanation of the differences between the results of the European and American observers. The training diet of American athletes usually contains about the same proportion of alkali forming foods as their non-training diet. The only dietary change which was made by our subjects during the entire experiments was that the men took 60 grams of gelatin a day for a period of 6 weeks during the middle of the training period. This caused no change in the properties of the blood. Figure 5 includes the mean values of CO_2 tension in basal alveolar air. There was an average decline of about 1.5 mm. Hg after training started which was in the opposite direction from the change noted by Herxheimer (1924) and by Ewig (1928). Since Bock and Field (1924), Bock et al. (1929) and Robinson (1938) have proved that resting alveolar air gives a close approximation of the arterial CO_2 tension and there was no change in the CO_2 combining capacity of the blood we may assume that the acidity of arterial blood did not increase in these men and therefore there was no increase either in CO_2 tension or acidity as a stimulus for resting respiration.

Exhausting work. Figure 1 shows the percentage changes of HbO_2 capacity and cell volume of blood and of protein in plasma in the adaptation from rest to exhausting work. In the initial test before training started the percentage increases in cell volume and in hemoglobin are the same. Increments of hemoglobin resulting from the work show no significant changes which are related to the training period. Increments in cell volume resulting from the work change gradually from 7.8 per cent in the initial test to 13.4 per cent in the final test. This discrepancy between changes in hemoglobin and in cell volume is accounted for by the fact that the men accumulated more lactic acid in work as the training period advanced (fig. 2). Dill, Edwards and Consolazio (1937) have shown that as the pH of blood decreases the cell volume increases. In the initial test the plasma protein increased by 18 per cent as the result of work. If we assume that as the plasma lost water to the tissues in this experiment no protein passed from the circulation, the actual increase in hemoglobin corresponded to the increment calculated from the concentrations of plasma protein and cells. This relationship confirms the observations of Dill, Talbott and Edwards (1930) on non-athletic subjects performing submaximal work. In subsequent tests during training the relationship was not so exact in our subjects, water loss from the plasma not being great enough to account entirely for the changes in hemoglobin.

Figure 2 shows, in 4 experiments, the effect of the exhausting run upon the blood lactic acid and alkaline reserve. It will be noted that the mean

values of blood lactate after the run increase progressively from 13 mEq. per liter before training to 17.9 at the end of the training period and that the alkaline reserve is correspondingly decreased in each set of experiments. Individual values of lactate and alkaline reserve, plotted in figure 2, show much wider variation than the resting values. Though there was a general increase in ability to accumulate lactate in these experiments the individuals were not capable of repeating their highest previous values every time they tried. The fact that the men became capable of utilizing the lactic acid mechanism more completely during the training period is one of the most interesting changes observed in this study. Since there was no increase in the amount of available base for buffering the acid, as indicated

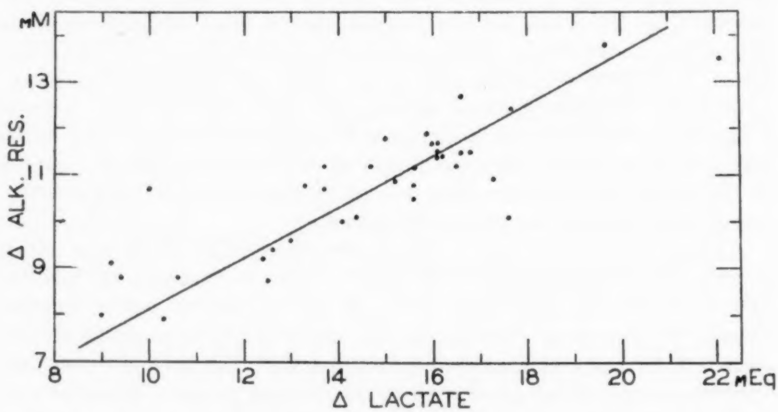

Fig. 3. Adaptation to the exhausting run. Blood lactic acid increases above the basal values (Δ-lactate) are plotted against the corresponding decreases in alkaline reserve (Δ-alkaline reserve) for the 36 individual experiments. The straight line calculated by the method of least squares indicates the relationship.

by basal alkaline reserve, the higher lactates accumulated after the runs during training made the reserve alkali lower than in the initial tests. The decrease below the basal level of alkaline reserve in the run was not equivalent to the rise in lactate, the latter being greater by about 2 mEq. per liter before training and by 5 mEq. after 6 months of training. As the lactic acid concentrations became higher in the later experiments more of it was buffered by base from proteinate and the pH necessarily became lower.

It is interesting to make a further analysis of the relation of blood lactate to alkaline reserve in work. Figure 3 shows, for the exhausting run, the individual blood lactic acid increases above the basal values (Δ-lactate) plotted against corresponding decreases in alkaline reserve (Δ-alkaline

reserve). In all but one of the 36 individual determinations the change in lactate was greater than the change in alkaline reserve. A straight line calculated by the method of least squares indicates that in this range of concentrations a change in Δ-lactate from 10 to 20 mEq. per liter corresponds to a change in Δ-alkaline reserve from 13.6 to 8.1 mM. Reports in the literature are controversial on this relationship. Mellanby and Thomas (1920) and Evans (1922) found that changes in alkaline reserve were smaller than corresponding additions of lactic acid to drawn blood. In Evans' data the difference was greater at physiologically high concentrations of lactate than at low concentrations. His results in the physiologically high range were very close to ours, a change in Δ-lactate of 10 mEq. corresponding to a change of 5 mM. in Δ-alkaline reserve. Barr, Himwich and Green (1923) found wide variations in 6 exercise experiments on men and that Δ-lactate was higher in only 2 cases. Dill et al. (1930) found that Δ-alkaline reserve was generally greater than Δ-lactate in exercising men where the blood lactate increased up to 6 mEq. per liter. Dennig et al. (1931) found the changes to be about equal in men at work where the blood lactate increased to about 10 mEq. The conditions of these authors were different from ours in two respects: their lactate values were lower, and in the last two papers the analyses were made by the method of Friedemann, Cotonio and Shaffer (1927) which tends to give lower recoveries of blood lactate than the method as revised by Edwards (1938) which was used in this study.

Figure 4 includes the mean blood lactate determinations after 9 sets of exhausting runs, including the 4 tests in which alkaline reserve was also measured. This curve shows the same increase of lactate with training which was described above. There is one break in the curve at the end of January which is probably related to the fact that the tests were made during examination week when the men were tired and nervous and for that reason were either unwilling to extend themselves or incapable of doing so as completely as in the preceding test. The mean blood lactate of 13 mEq. per liter in the initial test is very close to averages reported by Robinson et al. (1941) of initial tests on 83 non-athletic young men in similar exhausting runs on the treadmill.

The increased ability of the men to accumulate lactate in exhausting work was accompanied by a decline in the average alveolar CO_2 tension during work, an increase in the excess (above basal) O_2 consumed in the first 15 minutes of recovery, and no change in blood sugar concentrations (fig. 4). The lower alveolar CO_2 values in the later experiments were due to overventilation of the lungs associated with the greater concentrations of lactate. This accounts in part for the increase with training in the tolerance of the men for lactate. Probably improved circulation to the leg muscles also played a part by removing and distributing lactate faster so

that the organism as a whole could buffer more of it. There is also a possibility that the men underwent changes in sensitivity to acidosis and in determination and confidence. The greater rate of O_2 consumption in early recovery was probably associated with increased circulation, due in

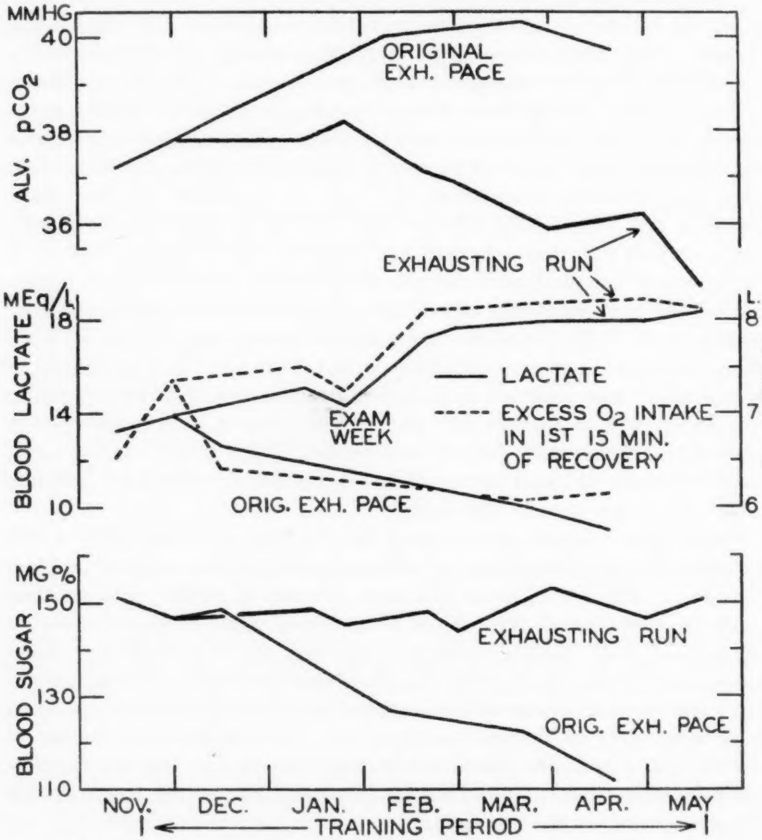

Fig. 4. The effects of training on the mean values of blood sugar and lactic acid 5 minutes after exhausting work, of alveolar CO_2 tension during the same work, and of excess O_2 intake (above basal) during the first 15 minutes of recovery. The same data are given for repetitions, during the training period, of the run at the same pace which was originally exhausting for the 9 men.

part to the influence of the high concentration of lactate in the body and in part to improvement of the circulatory mechanism with training. The fact that the blood sugar values did not increase along with lactate may indicate that the later runs were no more of a shock or stimulus to the

"emergency mechanism" than were the earlier runs when the accumulated lactates were lower but the men were not accustomed to them.

Not all of the subjects were able to complete 5 minutes of running at the speed and grade assigned in the initial test. The poorest runner required two months of training to accomplish this. At intervals this same 5-minute run was repeated even after it became submaximal and the men had all progressed to harder speeds and grades for their exhausting work. Figure 4 shows that after the 2nd test there was a rapid decline in blood lactate following this run from a mean of 13.7 mEq. per liter in December to 9 mEq. in April. This decline in lactate means that the men were able to complete the run with a decreasing percentage of the energy being supplied by anaerobic processes. It implies an increase in the maximal ability of the men to consume O_2 which is related both to improvement of circulation and of oxidative processes in the muscles, and perhaps an increase in skill which would decrease the total amount of energy required to maintain the pace. (Our data on O_2 consumption and efficiency will be presented in another paper.) Corresponding to the decline in lactate in repeated tests at this original pace there was a decline in the excess (above basal) O_2 consumed in the first 15 minutes of recovery though the curves do not follow parallel courses. The alveolar CO_2 tension increased from an average of 37.2 to 40.3 mm. Hg as the lactates become lower. This is in accord with the results of Schneider and Ring (1929) who have found that training increases the intensity of work which a man can perform before the concentration of CO_2 in expired air begins to drop. The blood sugar declined from 150 to 112 mgm. per cent indicating that the test was not eliciting the "emergency mechanism" so extensively in the later experiments.

Submaximal work. Figure 5 shows the effects of training upon the response to the standard walk and the 10-minute or moderate run. In the walk blood lactate and blood sugar were only moderately elevated and showed downward trends with training—blood sugar did not rise above the basal level in the late experiments. It should be remembered that the men were being trained for running and not for walking. We have found that distance runners after several years of training perform this walk with no elevation of blood sugar or lactate above basal levels. A low blood lactate in this work indicates a superior oxygen supply to the tissues. Alveolar CO_2 tension in the walk made no consistent change, remaining about the same as the resting values before and during training. The fact that alveolar CO_2 in the walk was the same as at rest is associated with the fact that no marked acidosis was developed—after 8 minutes of walking when the men had attained a steady state all energy for the work was supplied aerobically. The samples of alveolar air were collected near the end of the 15 minutes of walking. The absence of a training effect on

alveolar CO_2 in this work lends support to the assumption that the sensitivity of the respiratory mechanism to CO_2 is not affected by training.

In the initial moderate run blood sugar and lactic acid were both significantly elevated. Blood sugar declined rapidly with training and was only slightly above the basal value in the last 3 experiments. The decline of lactate was more gradual and at the end there was room for continued

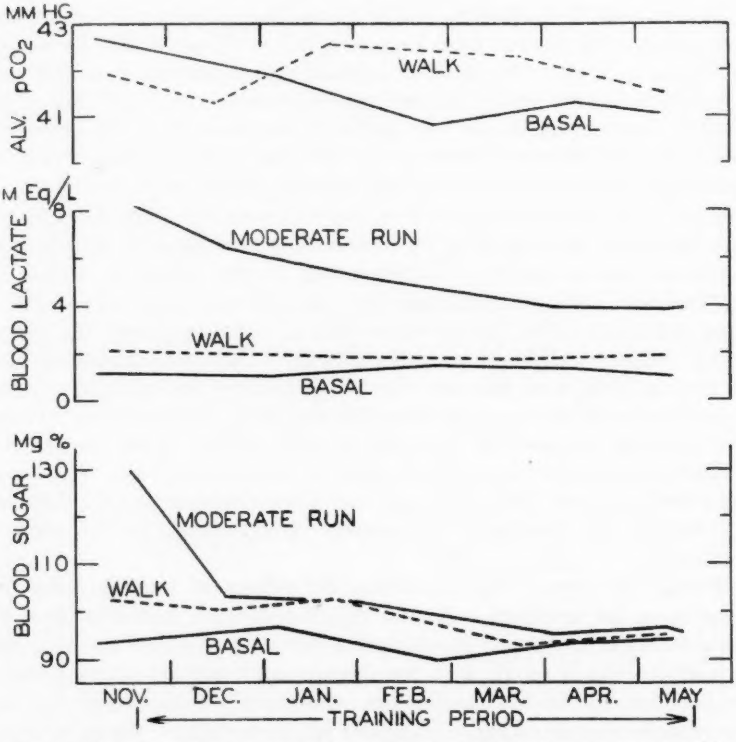

Fig. 5. The effects of training on the mean values of blood sugar, lactic acid and alveolar CO_2 tension in submaximal work.

improvement with further training. A decline of blood lactate with training in a less severe run than this has been previously noted by Edwards, Brouha and Johnson (1940). We have found that distance runners after several years of training perform this run with no elevation of blood sugar or lactic acid above resting levels. In this run, as in the walk, a low blood lactate indicates a superiority in O_2 supply to tissues. In the initial test the average O_2 requirement for maintaining the run was 90 per cent of the

average maximal capacity of the men for O_2 consumption as compared with 73 per cent at the end of the training period. A low blood sugar indicates that the "emergency mechanism" has not been elicited as it is when the organism is subjected to severe stress.

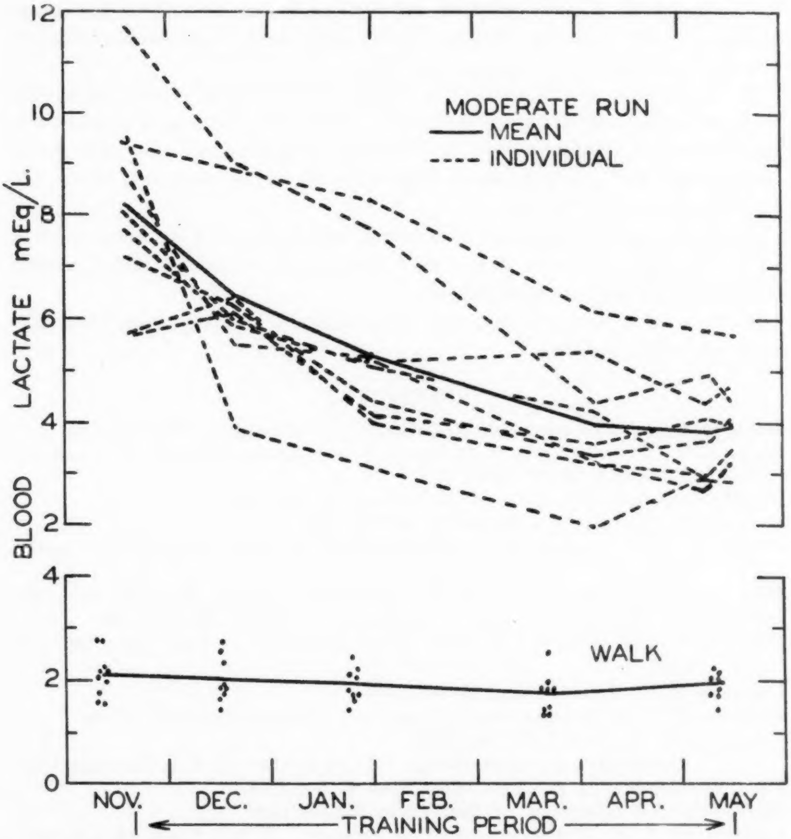

Fig. 6. The effects of training on the individual and mean records of blood lactic acid in two grades of submaximal work.

Individual variations of blood lactate in the walk and moderate run are shown in figure 6. The significant characteristic of the walk is the narrow range of variation. In the moderate run it is interesting to note the consistency with which the individuals improved. In the first 5 months there were only three individual tests in which a subject failed to do as well as

in his preceding test. This characteristic of the individual curves is due partly to the fact that the intervals between successive tests were long enough to allow significant training effects.

SUMMARY

1. Strenuous athletic training for 6 months did not affect the basal HbO_2 capacity, plasma protein, blood lactic acid, blood sugar, alkaline reserve or alveolar CO_2 tension in 9 men.

2. The ability of the men to accumulate lactic acid during anaerobic work increased with training; during the same work there were corresponding declines in alkaline reserve and alveolar CO_2 tension. The changes in blood sugar and HbO_2 capacity caused by the work remained about the same throughout training.

3. During grade walking blood sugar and lactic acid declined slightly with training. Alveolar CO_2 tension remained unchanged and at about the same level as the basal values.

4. In submaximal running the blood lactate and sugar declined significantly with training.

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CHEMICAL CHANGES IN THE BRAIN PRODUCED BY INJURY AND BY ANOXIA[1]

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The effects of cerebral injury and of cerebral anoxia on the cortical pH and electrical activity and on the concentrations of lactic acid, inorganic phosphate, phosphocreatine, pyrophosphate and "hexose phosphates" of the cerebral cortex have been investigated.

PROCEDURES. The cerebral hemishperes of cats anesthetized with nembutal or dial were exposed and electrodes suitable for the measurement of electrical activity and pH (1) were placed on the cortex. The electrical activity and pH were continuously recorded and when the character of either record indicated that significant alteration of the chemical pattern of the brain might be expected the area under observation was frozen *in situ* with either precooled metal blocks or directly with liquid air. The frozen tissue was then removed and kept in liquid air until chemical analysis was begun.

One gram of tissue consisting principally of grey matter was taken for analysis. Lactic acid and the phosphate fractions were determined by methods already described (2). The pyrophosphate fraction is believed to be almost entirely adenylpyrophosphate, while that labelled "hexose phosphates" includes hexose-6-monophosphate, hexose diphosphate and triose phosphates, if present, and possibly other compounds as well.

RESULTS. 1. *Effects of injury.* A small portion of the cerebral cortex (5 x 15 mm.) was frozen with a precooled metal block and removed. Somewhat later a sample was taken in the same way from an adjacent area. On examining the two samples chemically it was found that the second sample had a greatly increased lactic acid concentration. The results of several such experiments are summarized in table 1.

In a second group of experiments a portion of the cerebral cortex was frozen with liquid air and a block of the cerebral tissue was removed. At

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[2] Alexander Brown Coxe Memorial Fellow, Yale University, 1939–40.

a later time a homologous area of the contralateral hemisphere was sampled in the same way. It was found that the second sample contained greater amounts of lactic acid and of inorganic phosphate than did the first. The phosphocreatine content and the pH of the second region were both diminished. The acid shift in the contralateral hemisphere following the injury to the first hemisphere could be prevented if the respiration of the animal was increased at the time the injury was made, but essentially the

TABLE 1

Effect of freezing and extirpation of one area on the lactic acid content of an adjacent area

AREA	LACTIC ACID OF THE CORTEX				
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
	mgm./100 gm.	*mgm./100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*	*mgm./100 gm.*
First	38	28	37	56	31
Second	62	74	91	86	101

TABLE 2

Effect of freezing and removal of part of one hemisphere on the contralateral hemisphere

WEIGHT OF CAT	HEMISPHERE	RESP. VOL. AT 28/MIN.	pH	LACTIC ACID	INORG. PHOS.	PHOSPHO-CREAT.	PYROPHOS.	HEXOSE PHOS.
kgm.		*cc.*		*mgm. per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*
3.23	First	40	7.24	7	8.1	14.9	13.2	23.9
	Second	40	6.96	29	9.9	12.2	14.4	23.1
2.97	First	60	7.33	27	8.6	11.9	10.7	24.5
	Second	60	6.90	82	12.3	9.9	12.0	22.1
3.24	First	75	7.10	72	11.9	9.0	11.7	17.7
	Second	75	6.70	109	13.3	7.8	9.5	17.0
3.27	First	50	7.33	22	15.2	13.1	11.8	19.1
	Second	100	7.16	86	12.7	9.4	10.9	18.6
2.87	First	60	7.02	40	12.3	11.7	12.1	19.8
	Second	100	7.08	95	17.1	7.3	9.9	13.9

same changes occurred in the concentrations of the lactic acid and phosphate fractions. The results are summarized in table 2.

In a few experiments the pH electrode alone was used as an indicator of chemical change. It was found that either freezing or cutting of cortical tissue could produce changes in the chemistry of adjacent and more remote regions. While the quantitative effect was variable, the effect produced in the area under observation seemed to be dependent on the distance from the injured region and the extent of the injury. It was also noticed that less disturbance was produced to the rest of the cortex if the cerebral tissue was removed by "suction" rather than by cutting.

2. *Effects of reduced cerebral blood flow.* A differential blood flow to the two cerebral hemispheres of a cat was achieved by the cutting of one common carotid artery. As the animal bled from the cut vessel both the electrical activity and the pH of the ipsilateral cortex decreased rapidly. Both hemispheres were frozen simultaneously a few minutes later, at a time when the contralateral cortex began to show a slight reduction in its electrical activity. The chemical results of two such experiments are summarized in table 3. It is clear that the hemisphere with the decreased blood supply had a greater concentration of lactic acid and of inorganic phosphate and a lesser concentration of phosphocreatine than did the control.

3. *Effects of low oxygen.* Three series of animals were used. The first series served as a control for the second and third and gave the concentrations of various chemical fractions that obtain in a nembutalized

TABLE 3

Effect of a reduction of blood flow on the chemical pattern of the cerebral cortex. Left common carotid artery cut

HEMISPHERE	pH		LACTIC ACID	INORG. PHOS.	PHOSPHO-CREAT.	PYROPHOS.	HEXOSE PHOS.
	Before	After					
			mgm. per 100 gm.	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*
Right	7.21	7.20	30.9	11.7	11.5	13.4	14.8
Left	7.09	6.87	71.0	16.8	9.5	13.2	16.2
Right	7.03	6.97	30.9	13.4	9.0	12.8	15.8
Left	6.98	6.70	59.0	17.9	6.4	12.0	15.7

cat breathing air. The values obtained for the concentrations of lactic acid, inorganic phosphate and phosphocreatine agree with results presented by Avery, Kerr and Ghantus (3).

The second series included those animals which were allowed to breathe nitrogen (for 2 to 3 min.) until their cerebral cortices became and remained electrically silent for 20 to 30 seconds. At this time samples, obtained by freezing with liquid air, were removed for analysis.

Animals comprising the third series also breathed nitrogen. These, however, were artificially ventilated with air soon after the electrical activity of the brain had ceased, so that the period of electrical silence (20 to 30 sec.) was equal to that of the preceding series. The brains were frozen some two and a half minutes later at a time when the electrocorticogram had nearly returned to its pre-anoxic appearance. The chemical results obtained in these three series of animals are summarized in table 4.

Among the chemical changes to be noted are: 1. An alkaline shift in

pH during the period of anoxia in spite of an increased concentration of lactic acid. 2. The reciprocal changes in inorganic phosphate and phosphocreatine. 3. The "rebound" in the levels of inorganic phosphate and phosphocreatine during the recovery period.

TABLE 4

Chemical pattern of the cerebral cortex before, during, and after a short period of breathing nitrogen

EXPERIMENT NUMBER	BRAIN pH Before anoxia	BRAIN pH During anoxia	BRAIN pH After anoxia	LACTIC ACID	INORG. PHOS.	PHOSPHO-CREAT.	PYROPHOS.	HEXOSE PHOS.
				mgm. per 100 gm.	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*
43	7.37			23.3	10.5	11.3	19.9	27.1
55	7.30			16.8	11.4	13.1	12.4	17.0
41	7.23			13.5	11.5	13.5	21.1	22.2
39	7.19			8.9	8.4	13.4	15.4	24.6
54	7.18			20.2	11.1	14.6	11.0	10.5
36	7.18			16.3	12.2	14.6	15.6	21.1
45	7.00			16.0	11.6	12.4	26.5	21.1
40	6.98			20.3	11.9	11.3	22.4	25.2
Average.....	7.18			16.9	11.1	13.0	18.0	21.1
77	7.33	7.50		61.4	14.3	7.6	18.3	20.0
78	7.03	7.13		55.9	13.7	10.5	16.1	18.5
79				58.0	14.7	9.0	25.5	23.2
81	7.02	7.20		50.6	13.6	7.9	22.3	28.3
82	7.30	7.33		52.3	15.3	7.2	23.9	26.2
88	7.20	7.25		48.8	14.7	6.0	11.8	16.2
Average.....	7.18	7.28		54.5	14.4	8.0	19.7	22.1
83	7.29	7.48	7.36	16.9	7.1	14.7	25.4	24.9
84	7.28			31.8	7.6	15.6	21.9	30.9
85	7.24	7.32	7.40	31.6	6.6	15.3	27.6	29.0
87	7.16	7.21	7.28	17.0	6.7	15.4	9.5	18.5
89	7.32	7.35	7.29	28.6	7.1	11.0	10.7	14.9
Average.....	7.26	7.34	7.33	25.2	7.0	14.4	19.0	23.6

A "rebound" in the electrical activity following the anoxic period was also noted similar to that already described by Bremer and Thomas (4), and it may well be that these chemical and electrical phenomena are causally related.

Discussion. The fact that injury to one portion of the cerebral cortex can produce changes in the chemistry and presumably in the function of adjacent regions must be borne in mind in attempting to evaluate the

functional deficits resulting from a supposedly local lesion. Some of the chemical effects are undoubtedly due to unavoidable alteration in the cerebral blood flow, as the similarity of the results presented in tables 2 and 3 attests.

The pH shifts reported in the several experiments cannot be completely accounted for. The change in lactic acid is at times proportional to the observed pH change, as in the experiments reported in the first part of table 2, but lactic acid does not quantitatively account for the observed pH changes if the carbon dioxide tension is assumed to be maintained at a constant level. Simple calculations will show that the phosphate fractions contribute little to the acid-base shifts. Thus carbon dioxide is left as the most likely regulator of cortical acidity, though other as yet undetermined acid or alkaline substances may play a significant rôle in this regulation. The alkaline shift during nitrogen inhalation in spite of lactic acid increase is partly accounted for by the evident overventilation of the animals, but Ingraham and Gellhorn (5) have reported similar pH changes during anoxia with a constant rate of artificial respiration. The importance of blood flow as a regulator of cortical pH is undoubtedly due in part to the effect of blood flow on the rate of removal of carbon dioxide from the tissue.

Previous workers have given evidence that the phosphorylating glycolytic cycle, well known in muscle, can function in the brain *in vitro*. In this study the observed occurrence of phosphocreatine breakdown accompanying lactic acid formation during oxygen lack and the rapid resynthesis of phosphocreatine during recovery show that this mechanism is also operating in the brain *in vivo*. The findings reported here are very similar to those reported by others on muscle in the early stages of oxygen lack and of fatigue (6). Similar observations have also been made on peripheral nerve (7).

The rapid disappearance of lactic acid from the brain during recovery from anoxia seen in these experiments is most probably due to removal of the substance by oxidation, for previous studies have shown indirectly that the diffusion rate of lactic acid between blood and brain is slow (8, 9). It would appear likely that the level of lactic acid in the brain is regulated by metabolic conditions within the tissue, the formation and removal proceeding by a reversible reaction with the aid of the lactic dehydrogenase which is known to be present.

The electrical activity of the cerebral cortex is well known to depend upon the adequacy of its oxygen and its glucose supply. But the relation of intermediary metabolites to the electrical activity of the brain is unknown, and the present results do little to clarify the situation. If the oxygen supply is interrupted, the cortex becomes eletrically silent after a slight decrease of phosphocreatine has taken place and before adenylpyrophosphate has been altered at all. With oxygen present, greater changes

in phosphocreatine and lactic acid have at times been observed without loss of electrical activity. The conclusion is thus forced upon one that although breakdown of phosphocreatine does take place in brain as well as in muscle, the energy necessary for the maintenance of electrical activity of the brain cannot be obtained from the phosphorylating systems present, nor from anaerobic glycolysis by any route. It may well be that electrical activity is maintained by the oxidation of glucose through some pathway independent of the phosphate cycle.

SUMMARY

1. The cerebral cortex has been analyzed, after freezing *in situ* with liquid air, for the following constituents: lactic acid, inorganic phosphate, phosphocreatine, pyrophosphate and "hexose phosphates".

2. Injury or removal of one area of the cerebral cortex can result in marked chemical changes in adjacent and remote areas.

3. The concentrations of lactic acid and of inorganic phosphate are increased and that of phosphocreatine is decreased in conditions of anoxia resulting from breathing nitrogen or from reduction of cerebral blood flow. The two conditions differ in that the electrical activity disappears while the cortex is shifting in an alkaline direction in the first and disappears while the cortex is shifting in an acid direction in the second.

4. The electrical activity of the cortex is obliterated in conditions of anoxia before any detectable changes in the levels of adenylpyrophosphate or "hexose phosphates" take place.

5. During recovery from anoxic anoxia, lactic acid promptly decreases to its normal level in the brain and phosphocreatine is apparently resynthesized. The concentrations of inorganic phosphate and phosphocreatine tend to "overshoot" their original values, while the electrical activity is showing a rebound.

In closing we wish to express our appreciation for the aid and encouragement received from the late Prof. J. G. Dusser de Barenne, in whose laboratory the work was done.

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THE RESPONSE OF THE CEREBRAL CORTEX TO LOCAL APPLICATION OF STRYCHNINE NITRATE[1]

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While the physiological effects of strychnine on the central nervous system are sufficiently understood to make it serviceable in many investigations, a satisfactory theory of how the chemical effect is mediated has yet to be deduced. This paper presents certain observations which should be taken into account in the construction of any theory of the chemistry of strychnine action.

PROCEDURE. Two types of experiment have been performed. In the first, strychnine was applied to a small area (2 by 3 mm.) of the sensorimotor cortex of a cat (0.5 per cent solution of strychnine nitrate) or of a monkey (3 per cent strychnine nitrate). The electrical activity of such a strychninized area was then followed continuously while the blood pressure or the oxygen content of the blood was varied.

In the second type, one hemisphere of a cat was painted with a 3 per cent solution of strychnine nitrate, and the pH (1) as well as the electrical activity studied. Twenty minutes later, while the strychninized cortex was showing the characteristic electrical activity designated as "spiking" by Dusser de Barenne and McCulloch (2), the treated area and the homologous contralateral hemisphere were frozen simultaneously *in situ* with liquid air. The tissues were examined chemically by procedures already described (3, 4).

Five cats and two monkeys anesthetized with nembutal and dial respectively were used for the blood pressure and low oxygen experiments, and each animal was subjected to repeated short periods of reduced blood pressure or lowered oxygen supply. The blood pressure was reduced by so adjusting an artificial respiration machine that increased air pressure in the lungs was maintained. The level of the blood pressure was indicated

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[2] Alexander Brown Coxe Memorial Fellow, Yale University, 1939–40.

on a mercury manometer connected to a femoral artery. The oxygen content of the blood was lowered by allowing the animals to breathe nitrogen containing small amounts of oxygen (0 to 4 per cent).

Results. A short time after the local application of strychnine to the cerebral cortex the distinctive type of electrical activity (spikes) appeared. When the blood pressure was progressively reduced, the strychnine spikes decreased first in frequency and then in amplitude, finally disappearing altogether at blood pressure levels of 30 to 40 mm. of mercury. The "spontaneous" electrical activity was decreased by this procedure but was still present at a time when strychnine spikes were no longer seen. When the blood pressure was allowed to rise the spontaneous activity increased first; later the strychnine spikes reappeared and gradually returned to their former frequency and amplitude. Several of the cycles could be followed after a single application of the strychnine solution, showing that the initial disappearance was not simply a matter of the strychnine effect wearing off. Selected samples of the electrocorticogram obtained during one such cycle are contained in figure 1.

In one animal, a monkey, whose blood pressure was initially in the vicinity of 40 mm. Hg, strychnine spikes were not obtained following local strychninization until the blood pressure had spontaneously improved.

When the animals were made to breathe nitrogen containing small amounts of oxygen, the strychnine spikes were found to be more sensitive than the spontaneous activity to anoxia. The frequency of spiking decreased during the period of anoxia and finally the spikes disappeared at a time when the spontaneous activity was only slightly reduced in amplitude. When air was readmitted, the strychnine spikes returned, their amplitude and frequency increasing until they were again at their pre-anoxial level. It was possible to carry the animal through several such cycles following a single strychninization. In figure 2 are selected portions of a record of a single cycle.

Following the local application of strychnine, no pH changes have been observed at the site strychninized which could be ascribed with certainty to either the strychninization itself or to the intense electrical activity that follows. Similar findings with respect to pH have also been reported by Jasper and Erickson (5).

A comparison of the chemical analyses of a strychninized and an untreated hemisphere shows that there are no significant differences in the concentrations of inorganic phosphate, phosphocreatine, adenylpyrophosphate or "hexose phosphates" of the two areas. As can be seen in table 1, there may be a slightly greater concentration of lactic acid in the strychninized hemisphere, but the increase is so slight that a large number of animals would be necessary to establish the validity of the increase, if any.

Discussion. It is apparent from the results reported here that strychnine spikes are more susceptible to a reduced supply of blood or oxygen than is the normal electrical activity. The production of strychnine spikes apparently will take place only if oxygen is present at a somewhat higher

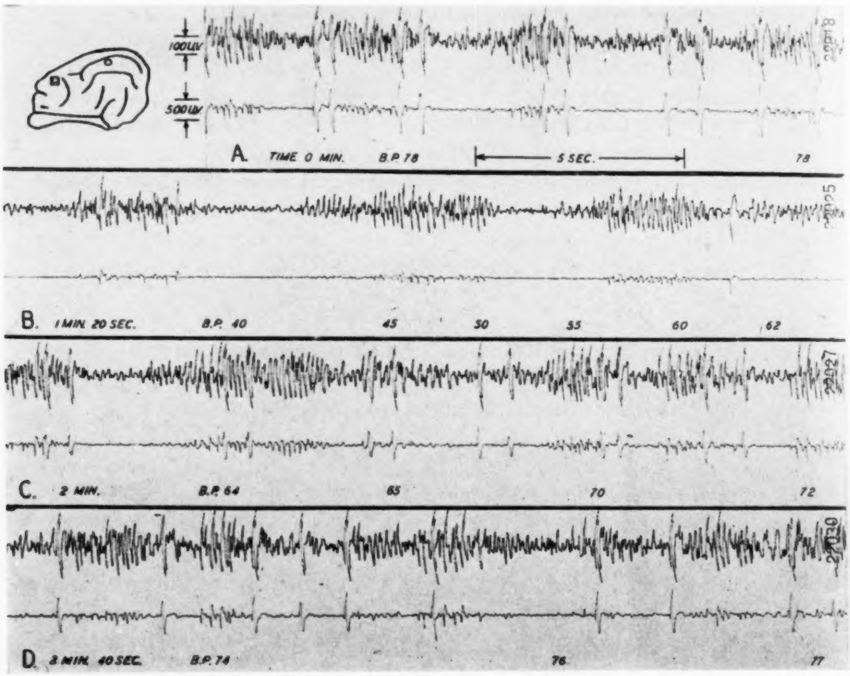

Fig. 1. The differential effects of low blood pressure on the "spontaneous" electrical activity and the strychnine "spikes" of a nembutalized cat. The two recordings were taken from the indicated areas of the brain at different amplifications in order to bring out the background activity in the upper record and the spikes in the lower. These records were obtained with a Grass electroencephalograph. The time and the blood pressures are recorded on the individual records. Blood pressure was reduced by raising the intrathoracic pressure. □ in the inserted diagram indicates the strychninized area. ○ indicates the electrode placement.

Note the disappearance of the strychnine spikes at low blood pressures in B and the diminished amplitude during recovery in C and D while the background activity is affected much less.

partial pressure than is necessary to maintain the "spontaneous" electrical activity.

It is somewhat surprising that such apparently intense electrical activity can appear in one portion of the brain without producing more distinct

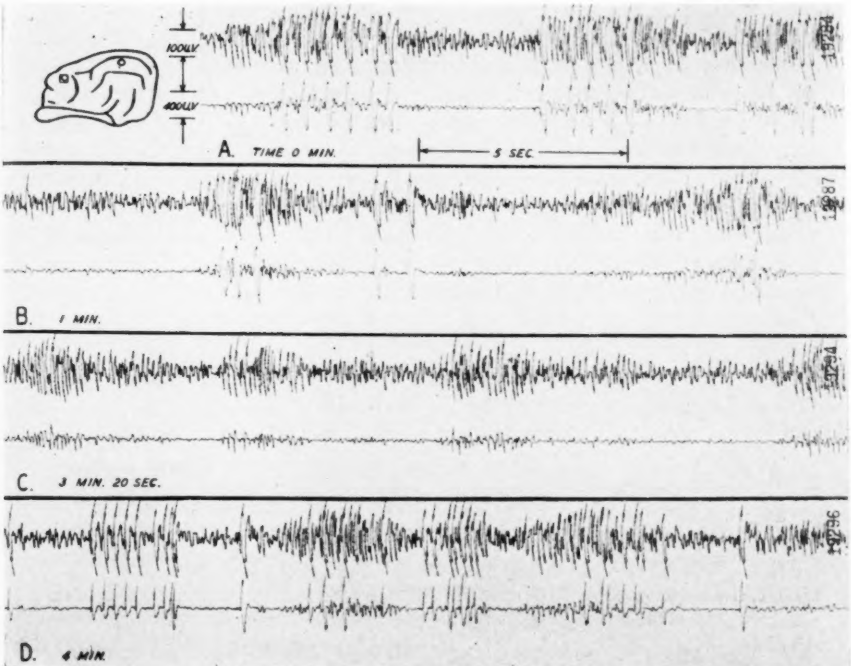

Fig. 2. The differential effects of low oxygen on the "spontaneous" electrical activity and the strychnine "spikes" in a nembutalized cat. Legends as for figure 1. In A the animal began breathing 4 per cent oxygen in nitrogen. In C the animal was returned to air.

Note decreased frequency of the spikes in B, the absence in C, and the return in D, while the background activity is hardly affected.

TABLE 1

Effects of local application of strychnine on pH, lactic acid and phosphates of the cortex

HEMISPHERE	pH		LACTIC ACID	INORG. PHOS.	PHOSPHO-CREAT.	PYROPHOS.	HEXOSE PHOS.
	Before	After					
	Application of strychnine to one hemisphere						
			mgm. per 100 gm.	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*	*mgm. P per 100 gm.*
Normal.......	7.20	7.18	20.2	11.1	14.6	11.0	10.5
Str............	7.10	7.15	23.2	12.3	15.4	11.9	12.7
Normal.......	7.25	7.30	16.8	11.4	13.1	12.4	17.0
Str............	7.15	7.17	20.3	10.0	12.1	11.0	18.7

chemical differences between that region and the untreated portion. The latter findings would be understandable, however, if the supposition is made that strychnine does not stimulate cells to greater activity, but rather synchronizes such activity as the cells possess. (See Bremer (6) and Gerard (7).) It might be supposed that if large numbers of cells were stimulated the products of their increased metabolism would modify the chemical pattern of the tissue, whereas if only synchronization of existing activity is effected, the chemical state of the tissue would remain essentially unaltered. This, however, can only be a supposition, for our present chemical methods are very crude and incomplete, and do not in any sense approach the recording of electrical activity in delicacy.

SUMMARY

1. Strychnine "spikes" are more sensitive to anoxia than is the "spontaneous" electrical activity of the cerebral cortex.

2. The application of strychnine to the cerebral cortex produces no significant changes in the pH or in the concentrations of inorganic phosphate, phosphocreatine, adenylpyrophosphate or "hexose phosphates".

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MEASUREMENTS OF INTRAMYOCARDIAL PRESSURE[1]

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Using the hypodermic manometer Gregg, Eckstein and Fineberg (1) showed that left ventricular pressure exceeding aortic pressure (as reported by Hamilton, 2) is recorded only when the needle tip does not lie free in the ventricular cavity. Since, by inspection, it was known in most instances that the needle opening was in a myocardial pocket of fluid it seemed a possible means of quantitating intramural pressure. Accordingly, these experiments were extended and attempts were also made to measure myocardial pressure through imbedded arterial segments (unpublished observations). Generally such pressure pulses exceeded the aortic systolic, but due to technical difficulties these measurements were not believed to demonstrate crucially that intramyocardial pressure exceeds ventricular during systole. Johnson and DiPalma (3) by use of imbedded arterial segments have recorded pressure pulses from the myocardium exceeding aortic pressure and believe they are an exact measure of intramyocardial pressure. Since these experimental findings are interpreted differently we have reinvestigated the subject.

Methods. The chest and pericardium of dogs were opened under sodium pentabarbital anesthesia and under artificial respiration. The blood was made non-coagulable by a mixture of heparin (100 units per kilo) and pontamine fast pink (200 mgm. per kilo). Attempts were then made to evaluate intramyocardial pressure in the following ways: 1. The systolic and diastolic pressures in a vessel segment closed at its peripheral end and imbedded in the left myocardial wall were determined in a manner similar to that of Johnson and DiPalma (3) and compared with the existing aortic or left ventricular pressure. 2. The phasic rates of flow through such imbedded vessel segments were determined by the orifice meter (Gregg and Green, 4) with blood led from the aorta or left ventricle, or with blood or Locke's solution led from the constant pressure meter (Green and Gregg, 5). 3. Pressures were recorded from intramyocardial

[1] The expenses of this investigation were defrayed by a grant from the Commonwealth Fund.

pockets of mineral oil, glycerine, Locke's solution and heparinized blood placed at varying depths.

RESULTS. *Recordings from imbedded vessel segments.* A pressure manometer was attached to one end of a segment by a cannula, the tip of which was generally, although not always, buried in the myocardium, while the other end of the imbedded segment was ligated at the epicardial surface. Pressures were then recorded under various distending intravessel pressures. These experiments confirm the findings of Johnson and DiPalma (3) that the pressure pulse generally exceeds the aortic or left ventricular pressure. However, when the aortic pressure is essentially constant at 90/74 mm. Hg an increase in the diastolic internal distending pressure from 84 to 204 mm. Hg causes a very large increase in the pressure pulse from 120 to 196 mm. Hg (fig. 1-A and B). Similar results were obtained with rubber tubing of comparable wall thickness.

Further massive augmentation of the vessel segment pulse (up to four times the left ventricular pressure) is obtained by the simple expedients of pulling the thread attached to the peripheral end of the segment so that it is maintained at an increased length (records not shown) or by elevation of blood pressure through mechanical constriction of the aorta (fig. 1-C vs D). Such values greatly exceed any normal expectancy for intramyocardial pressure.

In some experiments, to minimize possible movements of the vessel segment, it was pulled over and tied to a small, rigid, fenestrated metal tube which was closed and tapered peripherally and attached to the manometric system. This procedure did not alter the pressure relationship recorded in the myocardium and aorta.

Blood flow through an imbedded vessel segment. If intramyocardial pressure exceeds aortic systolic, as the preceding experiments suggest, then blood piped from the aorta under its normal pressure head into a deeply imbedded carotid segment (open at its peripheral end) should cease to flow during at least part of systole. Actual measurements of such phasic flow by the orifice plate meter (Gregg and Green, 4) interposed between the segment and the aorta may show at times a sizable systolic flow. Figure 1-E is a record from an experiment in which the minimal rate of systolic flow approximates 12 cc. per minute. Similarly, when Locke's solution held under a slowly declining pressure is led through the segment from the constant pressure meter of Green and Gregg (5) there may be a systolic flow of approximately 14 cc. at infusion pressures 8 mm. Hg less than aortic diastolic (fig. 1-F). These experiments indicate that, as measured by this method, the systolic resistance to coronary flow may be considerably less than aortic systolic pressure.

Recordings from myocardial fluid pockets. These pockets were produced by injecting oil, Locke's solution or heparinized blood under pressure into

the muscle wall of the left ventricle. The pulses were recorded from these pockets with hypodermic needles or fenestrated and tapered cannulae to

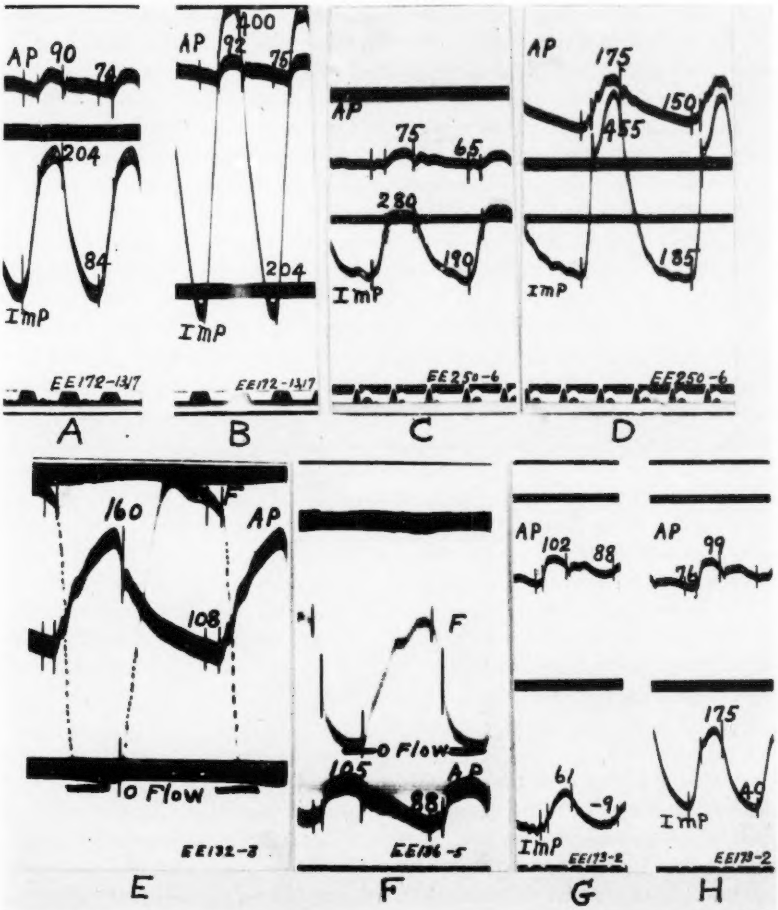

Fig. 1. A, B—records illustrating pressure pulses recorded from arterial segment imbedded in left ventricular wall at different diastolic segment pressures. C, D—records showing comparative effect of aortic constriction on aortic blood pressure and intrasegment pressure. E, F—records using orifice plate meter showing phasic blood flow through artery segments imbedded in myocardium of left ventricle (E with blood from carotid; F with Locke's solution from infusion chamber). G vs. H—records of pressure taken from myocardial pockets of blood in left ventricular wall and showing the effect of elevation of diastolic pocket pressure. AP—aortic pressure; IMP—intramyocardial pressure taken through vessel segment or fluid pocket. F—coronary inflow calibrated at top of light beam. Time—⅕ second.

prevent any possibility of occlusion. Figures 1-G and 1-H show that, as the diastolic pressure in a myocardial pocket of heparinized blood is increased during diastole from −9 to 40 mm. Hg, the resulting pulse rises from 70 to 135 mm. Hg, with a fairly constant aortic pressure. The same results were obtained with the other substances. In these experiments it was never possible to demonstrate the tremendous excesses of pressures recordable from the myocardium with vessel segments.

Discussion. From the foregoing experimental work it is evident that pressure pulses in excess of aortic or left ventricular pressure can be recorded from well imbedded vessel segments or from myocardial pockets of fluid. However, since with vessel segments *1*, the intramyocardial pressure thus recorded may be so high (two to four times the aortic pressure) and may undergo further massive augmentation when the segment is stretched or the blood pressure is raised; *2*, a systolic flow of blood may occur through such a segment when the central end of the segment is connected to the aorta for blood source, and *3*, in myocardial pockets the pressure pulse may increase greatly with the diastolic distending pressure, it is not believed that such methods faithfully record the true pressure existing in the intramyocardial space.

Experiments were then devised to find out if possible what artefacts contributed to the production of these myocardial pressure curves. It was felt that the vessel segment per se or movements and distortion of the segment induced either by fluid movement or myocardial impact might affect the pressure transfer. Accordingly, the hydrodynamic principles involved in the transmission of pressure through the walls of elastic tubes were studied *1*, with a mechanical model; *2*, with pressures recorded from small rubber bags, vessel segments and rubber membranes inserted into the ventricular chamber, and finally *3*, with pressures recorded from the segments protected by a fenestrated metal cap or by a fenestrated and retractable metal sleeve.

Pressure transfer in a schema. Two possibilities suggest themselves as to why the vessel segment per se might limit pressure transfer through it. 1. In such a recording system, which is not isometric, any membrane exerting tension and interposed between a manometer and the pressure source to be measured prevents 100 per cent pressure transfer. This is predicted by the equations:

$$\text{(A)} \quad \text{Pressure transfer} = \frac{-T}{r} + \frac{T'}{r'} + P'$$

in which P' is the applied external pressure, T and T' are the wall tensions and r and r' are the radii. Therefore, pressure transfer is complete only if $\frac{T}{r}$ equals $\frac{T'}{r'}$. But

$$\text{(B)} \quad \frac{T}{r} = A_0(1 - r_0/r) \quad \text{and} \quad \frac{T'}{r'} = A_0(1 - r_0/r')$$

in which r_o is the resting radius and A_o is a constant. Hence, since r' is less than r, $\frac{T}{r}$ will be greater than $\frac{T'}{r'}$ and pressure transfer will not be complete. 2. The volume/pressure ratio in a vessel segment is not constant but decreases (especially rapidly at higher pressure levels). This must mean that pressures transfer into the segment from an outside source, for equivalent pressures applied, becomes progressively less at higher distending pressures.

These predictions of less than 100 per cent transfer are demonstrated experimentally in the data in figure 3 obtained by use of the schema in figure 2. The schema consists of a rubber bag, tube or vessel segment, C, placed in a Lucite chamber, A. The elastic structure and chamber were filled with Locke's solution or blood, and various pressures placed in both

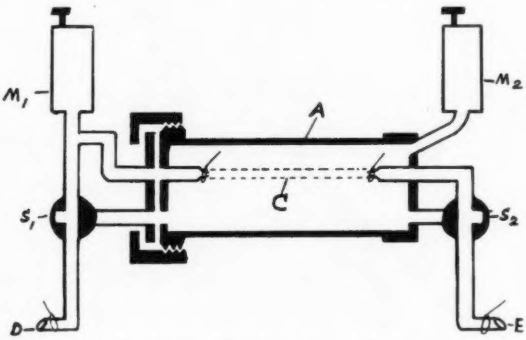

Fig. 2. Apparatus to determine extent of pressure transfer through vessel segments, rubber tubes and membranes under static and dynamic conditions. A—Lucite chamber; C—artery segment; M_1 and M_2—pressure manometers to determine pressure inside and outside segment; S_1 and S_2—stopcocks to direct pressures and blood flow through segment and chamber. D and E—cannulae.

were varied independently by turning appropriate stopcocks. The resulting pressure transfer was measured by Gregg pressure manometers (1, 6) M_1 and M_2. In any one experiment in which the segment was filled with fluid and the external pressure raised in successive increments the extent of such transfer was roughly the same over wide ranges of internal and external pressures, but in different experiments pressure transfer varied from 80 to 100 per cent. (See plot in fig. 3.) The pressure loss is maximal at the higher levels of segment inflation and can be significant at all pressure levels.

When the external pressure in the chamber was created by a pump system (producing a pulse pattern approximating a left ventricular pressure curve) pressure transfer could be augmented greatly by so directing the chamber flow that the artery segment moved. In figure 3 points in areas F and G

in which segment moved are to be compared with points in areas D and E in which segment did not move. The actual increase in pressure transfer is directly related to the extent of artery movement and inversely related to the diastolic inflation pressure. Here at a constant diastolic inflation pressure of 152 mm. Hg segment movement increases pressure transfer from 85 to 120 per cent (areas D versus F).

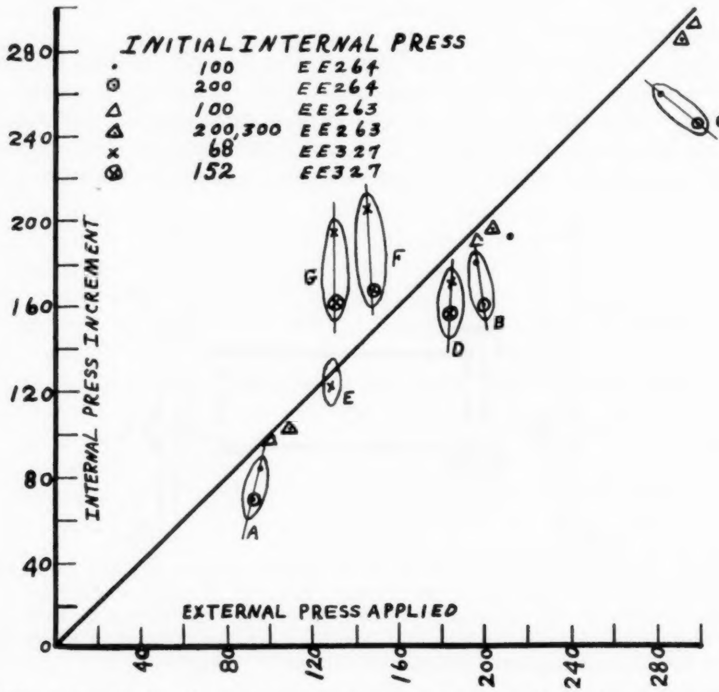

Fig. 3. Graph showing extent of pressure transfer through vessel segments using apparatus of figure 2. Initial internal pressure is intrasegment pressure before external pressure applied. Abscissa—mm. Hg external pressure applied. Ordinate—mm. Hg increase in intrasegment pressure due to applied external pressure. Solid line—theoretical curve for 100 per cent transfer.

Finally, when the chamber and segment are attached to the carotid artery of an anesthetized dog so that the blood pressure is transmitted to the chamber the pulse values recorded simultaneously from the external chamber and segment may be identical or may differ. An example of 93 per cent transfer is illustrated in figure 4-A. As a result of vagal stimulation the carotid pulse pressure is 95 mm. Hg (112/17), while the intrasegment pulse pressure is 88 mm. Hg (225/137).

Recordings from the ventricular cavity. To test further the accuracy of vessel segments as recording devices and under conditions which approximate those in the myocardial wall, the segments were attached to the manometric system by a metal cannula and then pulled into the left ventricular cavity (generally through the apex) by a needle and thread so that the whole segment was entirely free in the cavity. Its position was verified at the close of the experiment. If accurate, such closed segments should, at maximal inflation, give a pulse similar in contour, magnitude and timing to that obtained from the ventricle through an open cannula

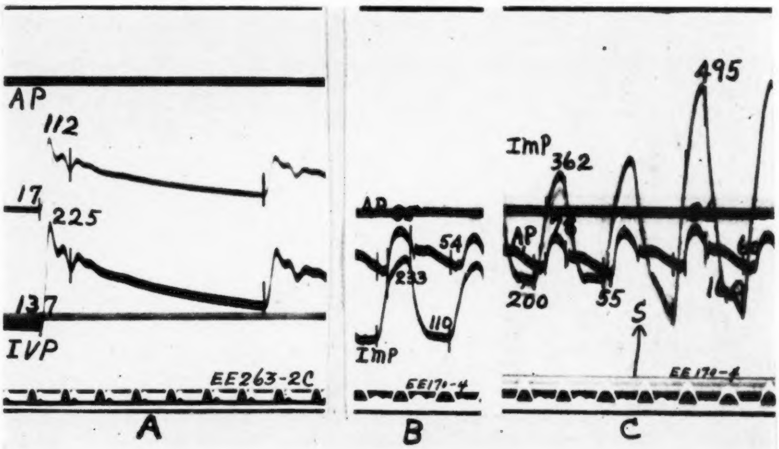

Fig. 4. A—record showing relationship between normal carotid pressure pulse, A.P., recorded from chamber A and pulse, I.V.P., after transfer through a carotid segment, C, in figure 2. B—record showing relationship between pressure pulse recorded from a closed artery segment in left ventricular cavity and the aortic pressure. C—record showing further augmentation of pulse in B by increasing diastolic intrasegment pressure and by stretching segment at S. AP—aortic pressure; IMP—intravessel pressure in left ventricular cavity; IVP—intravessel pressure in Lucite chamber; S—time at which vessel stretched; time—$\frac{1}{5}$ second.

or needle. In records figure 4-B and C elevation of the intrasegment pressure from 110 to 200 mm. Hg during diastole increases the pulse from 123 mm. Hg (already greater than aortic systolic) to 162 mm. Hg, while the aortic pressure remains at approximately the same pressure level. If now the aortic pressure is elevated by mechanical constriction of the aorta the increase of intrasegment pressure may double the change in aortic pressure (records not shown). In either instance, if the segment is now stretched by a cord extending through the ventricular wall and attached to its peripheral end, such pressure pulses can be increased still further.

In the last two pulses, figure 4-C, such a procedure augments the pulse from 162 to 335 mm. Hg. The maximum pressure thus far recorded from the left ventricular cavity by a closed vessel segment has been five times the ventricular pressure as registered through an open cannula.

Recordings from protected segments. To reduce the errors in pressure transfer thus revealed as caused by movement and changes in shape of the vessel segment the vessels were protected by placing over them loosely fitting fenestrated metal caps or metal sleeves, either of which could be removed as desired. Figure 5, A versus B, illustrates the effect on the pressure pulse of covering of the segment in the left ventricular cavity with a metal sleeve. Placement of the sleeve in B changes the pulse contour

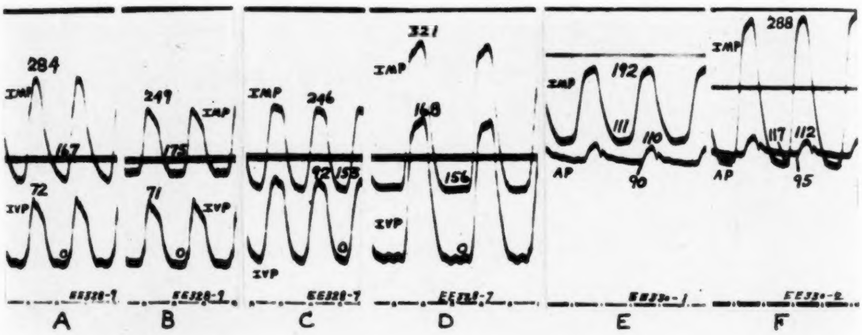

Fig. 5. A, B—records showing depression of intrasegment pressure in left ventricular cavity following addition of a protective fenestrated sleeve in B. C, D—records illustrating the comparative effects of mechanical constriction of the aorta on left ventricular pressure and intrasegment pressure when the segment is protected and in the left ventricular cavity. E, F—same as C, D but protected and unprotected segment in left ventricular wall. AP—aortic pressure; IVP—intraventricular pressure; IMP—intravessel segment pressure. Time—$\frac{1}{5}$ second.

to the ventricular pattern and reduces the pulse pressure value from 117 to 74 mm. Hg or to a value agreeing with the ventricular. One hundred per cent transfer may also occur when the intravessel distending diastolic pressure varies from 0 to 300 mm. Hg and when the blood pressure is raised by aortic constriction or synephrine injection (cf. fig. 5-C, D). Similarly, when a vessel segment protected by a sleeve is imbedded parallel to the left descendens and approximately one-half the depth of the myocardium, removal of the sleeve in F as compared to E, changes the pulse contour and greatly increases the pressure pulse value from 81 to 171 mm. Hg.

Protection of the segment thus reduces the error, and the agreement between the intraventricular pressure and intra-vessel pressure in figure 4

suggests that covered segments may give essentially correct values. However, such a possibility is remote. In the schema and in the ventricular cavity the pulse in the protected segment differs somewhat from the ventricular pressure curve, and predictions based on calculations and verified in many experiments (cf. fig. 3) indicate that pressure transfer can be incomplete. Finally, the fact that the pressure pulse recordable from an intramyocardial pocket of fluid or blood, although considerably greater than ventricular, increases progressively upon raising the pocket pressure, suggests that such a pulse is partially a function of the degree of stretch of local muscle fibres induced by the applied internal pressure. This error would presumably be present also in the imbedded vessel segments. Since these errors can not be removed the method of myocardial pockets of fluid or imbedded segments can not be used in a quantitative manner. However, since all these errors except the last can be quantitated for each segment in a schema the possibility remains for the use of either method in acute experiments as a rough index of directional changes in intra-myocardial pressure.

SUMMARY

Pressure pulses registered from myocardial pockets of fluid or by means of vessel segments imbedded in the myocardium of the left ventricle generally exceed by considerable amounts the aortic pressure simultaneously recorded. However, such pressures are in part artefactually produced and hence do not crucially demonstrate that intramyocardial pressure exceeds left ventricular pressure during systole. Our reasons for this belief are as follows: 1. The pressure pulse from an intramyocardial pocket of fluid increases progressively to values greater than aortic pressure upon raising the diastolic pressure in the pocket of fluid, and hence it is probably a function of the degree of localized muscle stretch induced by the applied internal pressure. 2. When blood from the aorta flows through an imbedded segment a systolic flow may occur. 3. Pressures recorded from closed vessel segments in the left ventricular wall or cavity may have two to four times the ordinate value of the intraventricular pressure, although the usual values are somewhat lower. 4. Protection of the segment in the cavity or wall by a loose fitting fenestrated cap or retractable metal sleeve reduces the recorded pressure pulse in the left ventricular cavity, in many experiments, to values approximating aortic pressure, and in the myocardium to values somewhat less than aortic pressure. 5. Theoretical and experimental evidence is given to show that pressure transfer through protected vessel segments is generally not complete. 6. However, pressure transfer exceeds 100 per cent when there is movement and distortion of the segment as a result of turbulent flow or mechanical impact. 7. Although the use of vessel segments may give directional changes, ex-

act quantitation of intramyocardial pressure is not yet believed to be a reality.

The authors wish to express their appreciation to Doctors W. Pritchard, T. Steege, and R. E. Shipley for their assistance in some of these experiments.

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THE VIABILITY OF SPERMATOZOA IN THE ABDOMINAL EPIDIDYMIS AND THE FAILURE OF MOTILE SPERMS TO FERTILIZE OVA

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Since the experiments of Hammond and Asdell (1926) upon the viability of spermatozoa in the male and female tracts, a good deal of work has been done which shows that the results obtained by them are of wide application among mammals. Bats alone do not seem to conform, as Guthrie (1933) has shown. Hammond and Asdell found for the rabbit that the fertility of sperm retained in the scrotal epididymis was 100 per cent up to 21 days, from 21 to 30 days it was 36 per cent, from 31 to 40 days, 20 per cent, and after this time the sperm were infertile. They retained their motility in one case, however, for 60 days. Probably part of this fertile time is taken up in the maturation of the younger sperm in the epididymis, so that the extreme time includes both the maturation period and the time during which the mature sperm retain their fertilizing ability. In this connection Young (1931) has found for the guinea pig an increase in the fertility of spermatozoa taken from the epididymis as the vas deferens is approached. This may account for 25 or more days of the life of the sperm of the guinea pig (Toothill and Young, 1931).

Young (1929) found that guinea pig spermatozoa remained fertile for up to 35 days when they were kept in the epididymis. They retained their motility, however, for 59 days. White (1933) found a fertile life for rat sperm up to 21 days, with a retention of motility for 42 days.

As the fertile life of rabbit spermatozoa in the female tract is limited to 30 hours (Hammond and Asdell, 1926), it is interesting to decide whether this reduced life is due to the higher temperature of the abdomen or to the motility of the sperm in the female tract, to mention but two of the possible reasons for the difference. Knaus (1932) has given data bearing on this point. He found that spermatozoa in the isolated abdominal epididymis were fertile for a maximum of 4 days, while in the isolated scrotal epididymis they retained their fertility for 12 days. The difference between the latter result and that of Hammond and Asdell, who found a fertile life of 40 days, is probably due to an earlier exhaustion of the supply

of sperm. Knaus was mating his rabbits daily, so that the one of three rabbits that maintained fertility to the twelfth day had mated seven times; when he was tested on the twenty-ninth day he was sterile. Motility was preserved in the abdominal isolated epididymis for a maximum of 12 days. He also determined the duration of fertility in the cryptorchid rabbit, in which the connection between the testis and the epididymis was undisturbed. His data are rather scanty and his rabbits were each mated several times. The maximum duration of fertility by this method was 7 days in one rabbit of three; at 6 days the fertility was two rabbits of three; while at 8 days no rabbit of three was fertile. He found motility in one rabbit of four at 14 days and in no rabbits of two at 15 and 16 days. From Knaus' data one concludes that the increased temperature without motility is instrumental in reducing both the fertile and potentially motile life. The duration of fertile life is reduced from 40 to 4 days by the increase of temperature in the absence of motility, while potential motility is reduced from 60 to 12 days' duration. These results were from the isolated epididymides. The reduction in the duration of fertility in spermatozoa from the testis was not quite so great. We have examined testes after a stay of 24 hours in the abdomen and have found that spermatogenesis has already ceased, so that in none of this work does the replenishment of the spermatozoa arise as a complication. Increased temperature, therefore, brought the fertile life down from 40 to about 7 days, and motility decreased this further to 30 hours.

We have now obtained more precise data on the duration of fertility and motility of spermatozoa in the testis and epididymis, and have determined the cause of the lack of connection between fertility and motility.

Experimental. We have used for the purpose of this work a number of male rabbits of Flemish Giant type of proven fertility and one year old or a little more. We have tried to use them as far as possible at one year of age. The method employed was to anchor the testes to the ventral abdominal wall with a loose suture through the fat body surrounding the caput epididymidis. Care was taken not to interfere with the blood supply in any way. The rabbits were then kept isolated until the day for testing, when they were given one or two matings. Later they were killed for motility studies. There was thus no possibility of semen exhaustion through previous matings. The results are summarized in table 1. Only bucks which actually mated are included in this table.

Our results agree closely with those of Knaus. We find that the extreme fertile life of the spermatozoa in the abdominal testis is 8 days compared with the 7 days found by him. Of 10 bucks mated to 15 does at 9 days, not one was fertile, and the same lack of fertility was obtained with 7 bucks mated to 14 does at 10 days. It may be added that we found no evidence of a reduction in the litter size in the fertile matings. A

large number of spermatozoa must have retained their fertility for a period and then lost it quite rapidly.

Table 2 gives the motility of the spermatozoa in these and other bucks used in this work. The procedure was to mince the epididymides and to add Ringer solution and examine on a slide or as a hanging drop. Each record represents a separate buck.

TABLE 1

Fertility of rabbits with abdominal testes

	DAYS IN ABDOMEN									
	1	2	3	4	5	6	7	8	9	10
No. of males tested	3	6	2	5	6	0	7	6	10	7
No. of matings	3	6	2	5	6		7	11	15	14
No. of fertile matings	3	4	1	4	3		1	3	0	0
No. of fertile males	3	4	1	4	3		1	2	0	0
Litter size of fertile matings	6.3	5.75	3.0	6.75	6.3		7.0	7.0*	0	0

* Average of two litters, the other litter was partly destroyed by the doe.

TABLE 2

Sperm activity in samples from the epididymides in the abdomen

DAYS												
1	2	3	4	5	6	7	8	9	10	13	14	15
T	T	0	M	T			T	F	S	0	0	0
M	M	M	M	M			T	F	T	0	0	0
M	M	M	M	S			F	S	T		F	0
M	M	M	M	0			T	F	F		0	0
M	M		0	T			T	T			0	0
M			M	T			T	S				0
M				T			T	T				0
				M				F				0
				M				F				0
				F				F				0
												0
												0

T = tremendous motility. M = motile. S = low motility. F = feeble motility. 0 = no motility.

It will be seen that motility ceased at about the fourteenth day. Twelve rabbits were tested on the fifteenth day without the detection of any motility. Only one specimen of five showed feebly motile spermatozoa at 14 days, so that this may be regarded as the extreme of motility. It was noted that the degree of activity of the individual spermatozoa and the number of motile spermatozoa agreed very closely. From table 1 it may

be seen that 4 bucks were sterile at 8 days. Of these, 3 had sperm with tremendous motility and 1 with feeble. This is additional evidence that fertility and motility are not parallel phenomena and that the lack of fertility at the extreme range of fertility is not due entirely to lack of evident vigor of the spermatozoa.

In order to decide why the motile spermatozoa did not fertilize eggs, 11 bucks with testes in the body cavity for 10 days were mated with does. The does were killed 9 or more hours after mating. Their reproductive tracts were searched for spermatozoa and for eggs. The results of the search were confirmed or corrected by sectioning parts of the oviducts. In two rabbits fertilization occurred, and examination of the bucks showed that the tail of the epididymis in each was in the inguinal canal. These records were rejected for this purpose. Records for the other 9 bucks and for the does follow:

1. Doe killed at 10 hours, sperm in the vagina and uterus, none in the oviducts.

2. Doe killed at 10 hours, no sperm found anywhere. In this case the epididymides were devoid of spermatozoa when the buck was killed at 15 days.

3. Doe killed at 10½ hours, a fair number of sperm in the vagina, a few in the uterus, none in the oviducts.

Doe killed at 12 hours, no sperm in the vagina, a few in the uterus, none in the oviducts.

4. Doe killed at 24 hours, a few sperm in the vagina, none in the uterus, none in the oviducts. Unfertilized eggs found.

5. Doe killed at 9 hours, no sperm found anywhere.

Doe killed at 24 hours, a few sperm in the vagina, a few in the uterus, none in the oviducts, 1 unfertilized egg found.

6. Doe killed at 12 hours, no sperm found in the vagina, a few in the uterus, none in the oviducts.

7. Doe killed at 16 hours, no sperm found anywhere, unfertilized eggs found.

Doe killed at 42 hours, no sperm or eggs found anywhere. The doe had ovulated. Dead spermatozoa were found in the epididymis at 15 days.

8. Doe killed at 16 hours, sperm found in vagina and uterus, none in the oviducts.

Doe killed at 48 hours, no sperm found anywhere, degenerating eggs found.

9. Doe killed at 16 hours, no sperm found anywhere, degenerating eggs found.

Doe killed at 48 hours, no sperm found anywhere, degenerating eggs found.

Dead spermatozoa found in the epididymis at 15 days.

No motile sperm were found in any of the females. From these results it is concluded that a few of the bucks may have failed to fertilize the does because no live sperm were present at the time of mating. This might apply to bucks 2, 7 and 9. For the remaining 6 bucks spermatozoa were found in the female tracts as far up as the uterus, but none in the oviducts and none were motile at the time of examination. In contrast, the two bucks with epididymides in the inguinal canal may be cited. Both these bucks fertilized eggs.

10. Doe killed at 10 hours, motile sperm in the uterus, non-motile sperm in the oviducts.

Doe killed at 24 hours, non-motile sperm found in the oviduct. Fertilized eggs found.

11. Doe killed at 14 hours, motile sperm found in uterus, no sperm in the oviducts.

Doe killed at 48 hours, non-motile sperm found in the oviducts. Fertilized eggs found.

Evidently the bucks with live spermatozoa were sterile because the sperms were able to travel as far as the uterus but lacked both the ability to travel further and the power to survive until the eggs were shed. This point now seems to be well established.

SUMMARY

The testes of adult male rabbits were anchored in the abdomen for varying periods. Spermatogenesis ceased within 24 hours. The extreme fertile life of sperms was 8 days for 2 males in 6 tested at this time. At 9 days none in 10 was fertile. No effect was observed on litter size at 8 days. Motility ceased at 14 days. Motile but non-fertile sperm failed to reach the oviduct or to survive to ovulation time after mating the bucks.

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THE EFFECT OF ETHER ANESTHESIA ON THE PLASMA VOLUME OF CATS

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The cat has often been employed in experimental studies of anesthesia, and many of the reactions of this animal to ether are well known. However, no previous report has been made concerning the effect of ether on the plasma volume in this species. It has been known for a long time that in dogs hemoconcentration regularly accompanies anesthesia with ether, and McAllister's (1937–1938) careful studies with the dye method showed that this is associated with and partly caused by an actual decrease in plasma volume, a fact subsequently confirmed by Bollman et al. (1938). Far less complete investigations in man indicate that ether anesthesia is associated with a much smaller reduction of plasma volume than occurs in the dog (Gibson and Branch, 1937; Ragan et al., 1939), but all of these determinations were complicated by the simultaneous use of other drugs or anesthetic agents. In a recent paper Barbour (1940) mentions two experiments on rabbits in which etherization was not attended by hemoconcentration, suggesting that no decrease in plasma volume occurred in these animals.

Methods. Fifteen experiments were performed on 13 normal adult male cats. The animals were given no food for 16 hours prior to experiments but were permitted water ad libitum. During the period of observation they were placed on their backs on a board and restrained by the application of loose ties to the legs. With gentle handling it was found possible to keep unanesthetized cats thus lying on their backs for long periods without struggling. Some cats actually purred throughout the period preceding anesthesia.

Plasma volume determinations were made on the unanesthetized animal with the blue dye T-1824 (Gregersen et al., 1935–1939), the procedure being essentially the same as that described by Hamlin and Gregersen (1939). The medial aspects of both hind legs were shaved, and the dye was injected into a femoral vein through a 24-gauge hypodermic needle. At 10 to 20 minute intervals 1.5 cc. blood samples were drawn without stasis from the opposite femoral vein. After a control period averaging

100 minutes etherization was started. The cat remained on the board, and ether was administered from a cone applied over the nose and mouth. By starting with low concentrations of ether in the cone, induction was accomplished with a minimum of struggling. Deep anesthesia was maintained for a period varying from 30 to 80 minutes. Blood samples were taken at regular intervals during the period of anesthesia, and changes in plasma volume were estimated from alterations in the disappearance curve of the dye. The serum protein concentration of each blood sample was estimated from the refractive index of the serum. In 4 experiments the serum protein concentration was also calculated from the serum specific gravity determined by the falling drop method. The values obtained by the two methods were in close agreement. In 5 experiments hematocrits were determined on heparinized blood using 1 cc. Wintrobe tubes centrifuged for 45 minutes at 3000 rpm.

TABLE 1

CAT	DATE	WEIGHT	PLASMA VOLUME		DURATION OF ANESTHESIA	MAXIMUM PER CENT CHANGE IN		
						Plasma volume	Serum protein	Hematocrit
		kgm.	*cc.*	*cc./kgm.*	*min.*			
1	2/18/38	2.95	138	46.8	30	0	0	
2	6/ 2/38	3.92	158	40.3	40	+6.4	−7.7	
3	6/ 7/38	3.36	164	48.8	30	0	0	
4	6/10/38	3.05	156	51.2	30	0	0	
	6/27/38	2.90	158	54.5	40	0	0	
5	6/15/38	3.16	208	65.8	60	0	−6.0	
6	6/17/39	3.29	133	40.4	45	0	0	
7	6/20/39	3.00	136	45.3	30	0	0	
8	6/23/39	3.30	137	41.5	80	0	0	
9	6/24/39	3.35	115	34.3	75	0	−6.5	0
10	6/27/39	3.30	132	40.0	50	0	0	+2.5
11	6/28/39	3.27	132	40.4	65	+5.5	−5.0	0
12	6/21/39	3.40	190	55.9	60	0	0	
	7/28/39	3.00	142	47.3	60	−7.4 to +8	+4 to −8	+4.7
13	12/20/39	3.86	173	44.8	50	0	0	0
Mean.................			151.5	46.5				

Results. The results of the 15 experiments are recorded in table 1. In 12 experiments the dye curves revealed no change in the plasma volume during anesthesia, and in all except two of these the serum protein concentration remained constant throughout the procedure. In the two exceptions (cats 5 and 9) there was a progressive fall in serum protein, but in one of these the decrease began before anesthesia was started.

Three cats showed alterations in plasma volume during etherization,

coincident with the beginning of anesthesia. Two of these animals gave definite increases in the volume of plasma. In one the increase was 6.4 per cent above the pre-anesthetic value and persisted for 40 minutes, the duration of the experiment. Another cat showed an increase in plasma volume of 5.5 per cent, but during the hour of anesthesia the plasma volume gradually approached the control value. A third cat responded to ether first with a decrease in plasma volume amounting to 7.4 per cent of the original value, but at the end of 40 minutes the plasma volume had been restored to its control level, and after 20 minutes more an 8 per cent increase had occurred. The early hemoconcentration in this experiment cannot be attributed to muscular activity during the induction period. The cat was remarkably quiet and was anesthetized with absolutely no struggling.

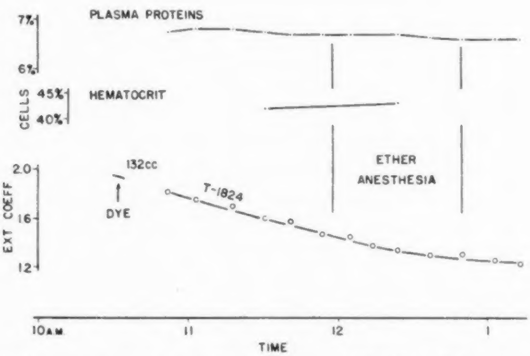

Fig. 1. Experiment on cat 10, weight 3.30 kgm. (6/27/39). Ether was administered for 50 minutes at the time indicated. The gradual flattening of the dye concentration curve is usually seen in unanesthetized cats and is a result of a decrease in the disappearance rate of the dye. No change in plasma volume is indicated by either the dye or protein concentration curves, while the hematocrit shows a slight but hardly significant rise.

In those experiments in which hematocrits were determined (table 1), one reading was taken during the pre-anesthetic period and another from 20 to 60 minutes after etherization had begun. One animal (cat 12) showed an increase in hematocrit of 4.7 per cent after 50 minutes of anesthesia, at a time when the dye and protein concentrations indicated that an increase in plasma volume had occurred. The other readings were within 2.5 per cent of the control value.

Discussion. The experiments clearly demonstrate that in the cat ether anesthesia does not cause a decrease in plasma volume. In this respect, therefore, the cat differs from the dog. According to the experiments of McAllister, etherized dogs showed an average reduction in plasma

volume of 11 per cent. The mechanism by which ether anesthesia leads to a significant reduction of the plasma volume of most normal dogs is not thoroughly understood. Root and McAllister (1939, 1940) have shown that the effect may be abolished by sympathectomy or by section of the spinal cord in the cervical or upper thoracic region. The change in plasma volume appears in some way to be dependent upon the integrity of the sympathetic system and its supra-segmental control. Hence it would seem reasonable to suppose that fluid is lost from the plasma as a result of altered pressure relationships in the capillaries incident to vasomotor changes. And yet it is interesting to note that Gregersen (1940) has demonstrated that the reflex vascular adjustments produced by clamping both carotid arteries in unanesthetized dogs fail to alter the plasma volume.

That ether causes a widespread stimulation of the sympathetic nervous system in the cat has been demonstrated by Bhatia and Burn (1933). The experiments of these investigators showed that the site of action of the anesthetic agent is on the central nervous system rather than peripherally. The studies of Root and McAllister indicate that the action of ether on the sympathetic nervous system of dogs is also central. In this regard, therefore, there is nothing at present to suggest that the basic action of ether differs in the two animals. The contrast in the responses of their plasma volumes to ether would seem more likely to depend upon differences in the result of sympathetic stimulation in the two species. Cannon (1939) has called attention to noteworthy variations between cats and dogs in the functions of the sympathetic system. Perhaps the influence of the sympathetic nervous system on the plasma volume is an example of such a species difference. The effect of generalized sympathetic stimulation on the plasma volume of cats has been subjected to study, but there is no agreement as to the result. Freeman (1933) injected adrenalin at a slow and constant rate into cats anesthetized with Dial and found that the plasma volume was markedly reduced. However, Hamlin and Gregersen (1939) repeated these experiments on unanesthetized cats and found that no decrease occurred.

A surprising finding in these experiments was the failure of the hematocrit values to rise sharply during the period of anesthesia. Bhatia and Burn measured spleen volume with a plethysmograph in decerebrate and spinal cats subjected to ether anesthesia. They found that etherization was associated with a prompt decrease in the volume of the spleen. In dogs, Barcroft and Florey (1929) and Hausner et al. (1938) have observed contraction of the spleen during anesthesia with ether. This splenic contraction is partly responsible for the hemoconcentration which occurs in dogs, for after splenectomy the rise in hematocrit reading during ether anesthesia is reduced to about half (Searles and Essex, 1936; McAllister, 1938; Bollman et al., 1938). In the present series, of the 5 cats on which

hematocrits were determined, the only animal showing a significant increase was also the only animal which gave evidence of a reduction of plasma volume. In these experiments it seems unlikely that a very intense contraction of the spleen could have occurred.

CONCLUSIONS

In normal cats ether anesthesia does not cause the reduction in plasma volume which has been reported in dogs and in man. The sharp rise in hematocrit reading seen in etherized dogs is also absent in the cat. The suggestion is offered that the differences in these responses of cats and dogs to ether may be attributed to differences in the action of their sympathetic nervous systems. These experiments again emphasize the danger of applying knowledge gained from one species to animals of another.

I wish to express my sincere thanks to Dr. Magnus I. Gregersen and to Dr. Walter S. Root for their helpful suggestions and criticism.

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RELATION OF SERUM AND MUSCLE ELECTROLYTE, PARTICULARLY POTASSIUM, TO VOLUNTARY EXERCISE[1]

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Since abnormal variations of potassium in the extracellular fluid are considered to exert a profound toxic effect on the heart, it is desirable to know if variations of potassium in extracellular and intracellular compartments limit the capacity of skeletal muscle to perform work. In a previous communication we demonstrated that within wide limits the amount of potassium in skeletal muscle did not effect the response of striated muscle to a tetanic stimulus (1). Since the testing of a muscle by a tetanus is not a satisfactory method of estimating the capacity of muscle for work, a less artificial method of testing work capacity was sought. In the present experiments the potassium of the skeletal muscle of rats was varied through wide limits by various means and the rat's ability to work was tested by having the animal swim continuously. It is obvious that in such experiments a failure to swim cannot necessarily be attributed to a decrease in muscle function, since many other factors might equally well explain the failure. If satisfactory work performances could be obtained in spite of abnormal variations of potassium in extracellular and intracellular fluids of the skeletal muscle, then an altered potassium in these fluids could not be accepted as an explanation for the failures.

Methods. Adult male rats weighing between 250 and 425 grams were used in all experiments. They were fed either Purina Dog Chow (stock diet) or a "low K" diet previously described (1). Food and water were given freely at all times. All rats were in apparent good physical condition at the time they swam.

The rats swam in a galvanized iron tank measuring 26 inches high by 20 inches in diameter filled with water at 37°C. to within four inches of the top. Since with no weight attached rats frequently could "loaf" and float, a five gram weight was attached by an elastic band to the base of the tail. The elapsed time each rat spent in the water was noted. A swimming performance was considered satisfactory if the animal swam con-

[1] Aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

tinuously for 60 minutes. Some rats were permitted to swim to the point of exhaustion. Exhaustion was determined by the inability of the rat to reach the surface of the water although it was making every attempt to do so. Occasionally rats submerged at the bottom for several seconds, evidently seeking a means of escape. By careful observation it was not difficult to determine when submersion was due to exhaustion and when it was due to other factors. Immediately upon termination of the swim the rat was etherized and blood withdrawn under oil from the abdominal aorta. Analyses of serum and muscle were made in the manner described in a previous communication (2). The muscles of the hind legs were the only ones used in the present study. The hearts of several rats were removed for pathological examination. Histological preparations were

TABLE 1

Serum and muscle electrolyte in exercised and resting rats with normal muscle potassium

GROUP	DIET	NUMBER OF RATS	AVERAGE TIME SWAM	SERUM: Per L. serum ultrafiltrate		SERUM: Per L. serum	MUSCLE PER 100 GM. FAT-FREE SOLIDS					
				Na	Cl	K	Na	Cl	K*	P	Prot.	H_2O
			minutes	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*gm.*	*cc.*
I	Stock	9	47	148.9	113.2	4.7	11.5	8.3	49.0	33.3	96.3	366.6
				±0.99	±0.99	±0.22	±0.45	±0.31	±0.45	±0.33	±0.95	±2.02
IA	Stock	13	0	146.9	113.0	4.0	9.99	7.2	48.8	33.4	92.3	340.5
				±0.74	±0.87	±0.21	±0.18	±0.15	±0.18	±0.35	±0.56	±1.90

* Corrected for extracellular K.

All concentrations represent mean result plus or minus the standard error.

studied after hematoxylin and eosin stains. Several rats received daily injections of desoxycorticosterone acetate.[2] The doses are indicated in the tables.

RESULTS. Table 1 shows the effect of prolonged exercise on the composition of serum and muscle in normal rats. The rats of group I swam for a period of from 15 to 110 minutes while those of group I A were killed as they were removed from the cages. In the serum the only significant change is the small increase in serum potassium from 4.0 to 4.7 mM per liter. In the muscle there is a definite increase in water with exercise. Since the increase in sodium and chloride of the muscle can account for sufficient extracellular water to explain the change in total muscle water, the amount of intracellular water per unit of fat-free solids is not appreciably changed by exercise. However, the increase in protein indicates a change in the solids—presumably a loss of some labile fat-free solid such

[2] We are indebted to Ciba Products, Inc., for the desoxycorticosterone acetate used in these experiments.

as glycogen. Since the data show no change in potassium and phosphorus per unit of fat-free solids, the change in solids is accompanied by loss of these elements.

The changes demonstrated in table 1 were apparently not related to the duration of the exercise—at least within the limits studied. Neither were they related to the apparent degree of exhaustion.

Table 2 shows the analyses of rats in which the muscle and serum potassium have been significantly increased by repeated hourly injections of KCl intraperitoneally. Analyses of rats in groups II A and III A are of resting rats and have been reproduced from a previous study (3) in order

TABLE 2

Serum and muscle electrolyte in exercised and resting rats with high muscle potassium

GROUP	DIET	NUMBER OF RATS	AVERAGE SURVIVAL TIME AFTER LAST INJECTION	AVERAGE TIME SWAM	SERUM: Per L. serum ultrafiltrate		SERUM: Per L. serum	MUSCLE PER 100 GM. FAT-FREE SOLIDS					
					Na	Cl	K	Na	Cl	K*	P	Prot.	H_2O
			minutes	*minutes*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*gm.*	*cc.*
II	Stock	7	19	8	145.9 ±3.28	121.3 ±1.48	9.4 ±0.48	8.4 ±0.36	7.7 ±0.17	50.9 ±0.41	33.6 ±0.39	94.4 ±0.43	366.6 ±0.75
II A	Stock	7	24	0	143.4 ±0.54	121.4 ±0.75	7.5 ±0.20	8.2 ±0.26	7.3 ±0.14	51.7 ±0.52	34.5 ±0.29	95.9 ±1.03	353.0 ±1.54
III	Stock	5	92	81	145.0 ±3.11	121.2 ±1.96	6.0 ±0.51	8.3 ±0.36	7.4 ±0.18	50.2 ±0.29	34.4 ±0.27	94.4 ±0.66	353.0 ±2.55
III A	Stock	5	78	0	145.2 ±0.86	120.0 ±1.14	5.5 ±0.23	8.7 ±0.30	7.2 ±0.19	49.5 ±0.36	33.2 ±0.18	94.8 ±1.28	346.3 ±2.82

* Corrected for extracellular K.

All rats in groups II and III injected with a total of 1.2 to 1.3 meq. KCl per 100 grams of rat in 6 hours.

All rats in groups II A and III A injected with a total of 1.1 to 1.4 meq. KCl per 100 grams of rat in 6 to 7 hours.

All concentrations represent mean result plus or minus standard error.

to show that the muscle potassium is significantly increased within 15 to 30 minutes after the last serial injection of KCl (see group II A) and returns almost to normal if the animal is not sacrificed until 60 to 90 minutes after the last injection (group III A). Groups II and III represent animals treated in a manner corresponding to groups II A and III A respectively except that they were exercised in addition to receiving injections of KCl. All rats in group II were rapidly exhausted by their swimming. The fact that they were exhausted did not apparently alter their electrolyte from that of the resting rats in the corresponding group II A. The only significant changes in electrolyte of the exercised rats of groups II and III from that of the resting rats in groups II A and III A are an increase in muscle water in both of the former and a higher concentration of serum potassium in group II than in II A. Unlike normal exercised rats there is no increase

in muscle sodium and chloride in the rats in groups II and III over those of the resting rats in groups II A and III A.

The rapidity with which injected potassium leaves the muscle, as demonstrated in table 2, makes it impossible to swim rats for 60 or more minutes and obtain values for muscle potassium at the highest levels. While the muscle potassium of rats in group III is significantly higher than in normal uninjected rats (see group I A), it is fairly certain that during the early part of their swim it was even higher, probably on the order of that of rats in group II. It is unlikely, therefore, that the high muscle potassium of rats in group II accounts for their failure to swim more than eight minutes. It is to be noted that the serum concentration of potassium of rats in group II is at the level at which electrocardiographic changes and toxic effects of potassium on the heart have been noted in cats (4). Presumably the concentration of potassium in the serum of rats in group III would have been found at or near this level had they been sacrificed earlier. Whether the rapid exhaustion of rats in group II was associated with a greater susceptibility to the toxic effects of potassium at this high serum concentration is a matter for conjecture and can not be ascertained from the data at hand. For the purposes of this study it is significant that satisfactory muscular work can be done in the presence of a high muscle potassium.

Owing to the relatively rapid fluctuations of potassium in the muscle when KCl is injected intraperitoneally it was not possible to ascertain what effect, if any, exercise may have on an already abnormally increased potassium in the muscle.

In order to determine if rats with abnormally low muscle potassium i.e., below 44 mM per 100 grams of fat-free solids, were able to swim satisfactorily, two methods for decreasing the muscle potassiums were used: the feeding of a diet low in potassium (3) and the injection of desoxycorticosterone acetate. We also sought to determine if exercise altered the muscle potassium at this low range. Consequently exercised rats that were fed the low K diet, group IV, have been compared with a similarly treated group of rats that were not exercised, group IV A. Similarly the rats injected with desoxycorticosterone acetate were divided into an exercised group, group V, and a resting group, group V A. The results are shown in table 3.

The rats in groups IV and V demonstrate that a low muscle potassium is not in itself a limiting factor in the satisfactory performance of work. Three of the seven rats in group IV swam a full 60 minutes without showing marked evidence of fatigue. The lowest muscle potassium observed (36.9 mM) was in a rat that swam 60 minutes. Comparison of the analyses of exercised rats in group IV with resting rats in group IV A shows that the muscle potassium per unit of fat-free solids apparently remains unchanged with exercise while a slight but significant increase in the concen-

tration of potassium in the serum of the exercised rats occurs. The increases in chloride and water of the exercised rats in group IV over those in group IV A are similar to those observed in exercised rats with normal muscle potassium (see group I) and apparently represent an increase in extracellular fluid in the muscle. The increase in muscle sodium, which was found to be significant in exercised rats in group I, is not so striking in the rats of group IV.

The effect of injecting fairly large amounts of desoxycorticosterone acetate subcutaneously into normal rats, group V A, is to decrease muscle

TABLE 3

Serum and muscle electrolyte in exercised and resting rats with low muscle potassium

GROUP	DIET	NUMBER OF RATS	AVERAGE TIME SWAM	SERUM: Per L. serum ultrafiltrate Na	SERUM: Per L. serum ultrafiltrate Cl	SERUM: Per L. serum K	MUSCLE PER 100 GM. FAT-FREE SOLIDS: Na	Cl	K*	P	Prot.	H_2O
			minutes	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*gm.*	*cc.*
IV	Low K 14 days	7	43	147.3 ±1.63	114.6 ±1.74	4.91 ±0.22	12.5 ±0.59	7.1 ±0.28	39.9 ±0.79	32.3 ±0.28	95.5 ±0.74	349.4 ±4.22
IVA	Low K 14 days	5	0	142.0 ±2.12	113.8 ±0.66	3.96 ±0.16	12.1 ±0.15	6.0 ±0.11	39.8 ±0.36	30.9 ±0.23	94.4 ±0.71	325.0 ±1.76
V	Stock	6	60	149.5 ±3.94	111.1 ±2.23	2.6 ±0.19	14.5 ±0.61	6.6 ±0.45	42.9 ±0.69	32.7 ±0.57	96.8 ±1.39	353.0 ±3.45
VA	Stock	11	0	152.0 ±1.30	107.9 ±1.48	3.7 ±0.08	14.6 ±0.39	6.3 ±0.08	40.5 ±0.82	31.7 ±0.24	95.5 ±0.59	328.0 ±3.59

* Corrected for extracellular K.

Group V—injected daily for 9 days subcutaneously with 2 mgm. desoxycorticosterone acetate in 25 per cent glucose.

Group VA—eight rats injected same as group V; remaining three rats injected with 3 to 4 mgm. desoxycorticosterone acetate in oil daily for 10 days.

All concentrations represent mean result plus or minus standard error.

potassium without altering the concentration of serum potassium. With the decrease in muscle potassium there is an increase in muscle sodium similar to that noted in rats fed a diet low in potassium (3). Both the serum and muscle chloride are significantly decreased. There is also a loss of muscle water. Contrary to the results obtained in rats fed the "low K" diet or the stock diet the muscle potassium increases and the concentration of potassium in the serum decreases with exercise (see group V). The changes in muscle sodium and chloride in the exercised rats of group V are negligible. The increase in muscle water, however, is significant. The failure to demonstrate an increase in muscle sodium in the rats in group V over that of the resting rats in group V A may have been

dependent on the fact that the expected increase associated with exercise was offset by the decrease in this cation that accompanies an increase in muscle potassium. It is to be noted that the increase in the muscle potassium of rats in group V is an absolute increase, since no change in muscle protein was observed over the control group V A. The work performance of the rats in group V was entirely satisfactory even though the muscle potassiums were below the recognized lower limit of the normal range (44 to 50 mM per 100 grams of fat-free solids).

Efforts were made to obtain satisfactory swimming performances with rats that had even lower muscle potassiums than those observed in table 3. Previous work had demonstrated that muscle potassiums as low as 30 mM per 100 grams of fat-free solids could be obtained by feeding rats on a low K diet for many weeks (3). Consequently a group of 7 rats that had subsisted on the diet low in potassium from 32 to 133 days were made to swim. One rat that had been on the diet 32 days swam for 52 minutes without fatigue, but all five of the rats that had been fed on the diet longer than 42 days became exhausted from swimming in 7 minutes or less. While this group of rats had muscle potassiums down to 30 mM in some instances, it is much more likely that pathological changes in the cardiac muscle accounted for their failure to swim well rather than the low muscle potassium. Microscopic studies[3] of the hearts of several of the rats that were so readily exhausted were made and pathological changes were found to be present. These changes correspond to those previously described by Thomas et al. in animals fed a low potassium diet (5).

Very low muscle potassium was also produced in seven rats by daily subcutaneous injection of 4 mgm. of desoxycorticosterone acetate for 30 days. The compound was suspended in a 10 per cent solution of glucose. The diet (Purina Dog Chow) contained adequate potassium. Three of the four rats which were placed in the tank were unable to swim longer than 20 minutes. The other one swam 60 minutes without apparent exhaustion. This rat showed a muscle potassium of 33.2 mM per 100 grams of fat-free solids, the lowest value found in any rat able to swim adequately. Serum potassium was 3.3 mM per liter.

The hearts of the rats given the excessive doses of desoxycorticosterone acetate were examined histologically except, unfortunately, the one which swam adequately. In each case, the myocardial fibres showed fairly extensive necrosis with displacement of necrotic fibres by connective tissue. The latter feature was not prominent since the necrosis apparently was not very old. The areas involved tended to be near larger blood

[3] We are indebted to Drs. M. C. Winternitz and H. M. Zimmerman of the Department of Pathology for their interpretation of the microscopic studies of the hearts of the animals fed the low K diet and also those injected with desoxycorticosterone acetate.

vessels but could be found in all parts of the heart. The lesions seemed to be comparable to those seen in rats fed a diet low in potassium for several weeks. Two of the rats which failed to swim showed ascites. It was felt that myocardial failure explained the inability of rats injected with excessive doses of desoxycorticosterone acetate to swim adequately.

DISCUSSION. The variations in the period of the swim of normal rats were found to be quite large. In two instances apparently normal rats with normal muscle potassium were exhausted within 10 minutes, two others were fatigued after 43 and 75 minutes and one rat swam 110 minutes without apparent fatigue. It seems justified to assume that 60 minutes of continuous swimming indicates a satisfactory performance.

The experiments clearly show that certain rats can swim for one hour with either a low or a high muscle potassium. Individual values as low as 33.2 and as high as 50.8 mM per 100 grams of fat-free solids were obtained at the conclusion of a swim of 60 minutes. Previous work showed that the muscle potassium of normal resting rats may vary from 44 to 50 mM per 100 grams of fat-free solids without being accompanied by a gross disturbance in either the serum or muscle electrolytes (3). While 50.8 mM of potassium per 100 grams of fat-free solids was the highest recorded value in a rat with a satisfactory swimming performance, the results in table 2 indicate that successful swimming was done by rats with considerably higher muscle potassiums. Although the muscle potassium of rats in group III was maintained at abnormally high levels for probably only 30 to 60 minutes, the results suggest that the similarly high muscle potassiums of rats with advanced adrenal insufficiency (2) are not per se the cause of the rapid muscle fatigue and weakness seen after adrenalectomy (6). While moderately low muscle potassiums, about 40 mM per 100 grams of fat-free solids, are compatible with satisfactory swimming performances in most instances, it is only rarely that rats can swim 60 minutes with much lower muscle potassiums. The myocardial injury associated with the very low potassium in the skeletal muscle, whether produced by a low K diet or the injection of desoxycorticosterone acetate, probably is the limiting factor.

Attention is directed to the fact that adequate muscular performances were obtained in group V although the concentration of potassium in the serum was very low (1.9 to 3.1 mM per liter). These rats received desoxycorticosterone acetate in amounts and over periods similar to the dogs reported by Kuhlman et al. (7). Our results suggest that myocardial injury rather than an abnormally low concentration of potassium in the serum is the cause of the "paralysis" and weakness noted in otherwise normal animals treated with desoxycorticosterone acetate.

The fluctuations in the level of serum potassium are of interest since these changes are sometimes used to indicate loss of muscle potassium

during exercise. Except in rats receiving desoxycorticosterone acetate, the exercising rats show a rise in serum potassium similar to that reported previously (8). Our data show that if potassium is lost from the muscle during exercise in the intact rat, it is lost together with sufficient fat-free solids to leave the potassium unchanged per unit of fat-free solids. Although we have no direct proof that non-protein fat-free solids were lost during exercise in the present experiments, such an occurrence would best explain the relative increase in protein per 100 grams of fat-free solids equivalent to four grams in group I. Expressing the relative increase in protein in terms of fat-free tissue (i.e., wet weight), the change in protein suggests a loss of fat-free solids equivalent to 0.5 per cent of the fat-free muscle. Within a few minutes after exercise, Flock and Bollman have demonstrated a loss of glycogen of about 0.3 to 0.4 per cent (9). If this glycogen is released together with sufficient potassium to keep the potassium constant per unit of fat-free solids, an exercising rat weighing 300 grams might free as much as 0.066 mM of potassium. This amount of potassium would raise the concentration of serum potassium 0.8 mM if confined to extracellular water and 0.3 mM if equally distributed throughout body water. Potassium might also appear in the serum from the liver since Fenn (10) has shown that glycogen is deposited in the liver together with potassium. Since the liver weighs about 8 grams the loss of 2 per cent glycogen would free about 0.02 mM of potassium. It is obvious that these two sources of potassium are more than sufficient to explain the rise in serum potassium accompanying exercise.

Fenn has postulated that muscular contraction is accompanied by a loss of potassium from the muscle per unit of solids (8). There can be no doubt, as Fenn points out, that electrical stimulation of an isolated muscle or group of muscles in an otherwise intact animal leads to a loss of potassium and a gain of sodium. Evidence that similar changes accompany *voluntary* contraction is not satisfactory. The only direct evidence for this view is submitted by Fenn (11). His conclusions are based essentially on differences between innervated and denervated muscle in resting as well as swimming rats. His data indicate that the "loss of potassium" with voluntary exercise may be due largely to a gain of potassium by the denervated muscle rather than to an actual loss from innervated muscle. To avoid this difficulty it would seem better to compare the muscles of rats that swam to those of rats confined to cages. Although the potassium of the innervated muscle from the exercised rats is lower than that of the resting rats, the difference is not statistically significant. The present experiments indicate that voluntary contraction is not accompanied by a loss of potassium beyond that associated with loss of glycogen or other fat-free solids. In fact, our work with rats injected with desoxycorticosterone acetate shows that potassium actually can enter the skeletal muscle cells during voluntary exercise.

SUMMARY

Within wide limits the amount of potassium in the muscle cells does not limit the capacity of rats to swim continuously for sixty minutes.

Concentrations of potassium in the serum that are abnormally low are not in themselves sufficient to produce "paralysis" or muscular weakness in normal animals.

No loss of potassium per unit of fat-free solids from striated muscle could be demonstrated after relatively long periods of voluntary exercise. An absolute increase in muscle potassium was demonstrated in exercised rats injected with desoxycorticosterone acetate.

Desoxycorticosterone acetate decreases the muscle potassium and increases muscle sodium in the normal resting rat. These changes involve an apparent replacement of intracellular potassium by sodium.

The increase in the concentration of potassium in the serum immediately following exercise was confirmed. The possible sources of this increase were discussed. The concentration of potassium in the serum of exercised rats injected with desoxycorticosterone is decreased.

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